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*Blood Pressure, Report of Joint Committee on Mortality of The Association of Life Insurance Medical Directors and The Actuarial Society of America, New York, 1925, pp. 10, 52.

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1. Fowler, W. M.: Current Therapy for 1951, Saunders.
2. Creskoff, A. J.: Am. J. Med. Sci., November, 1950.
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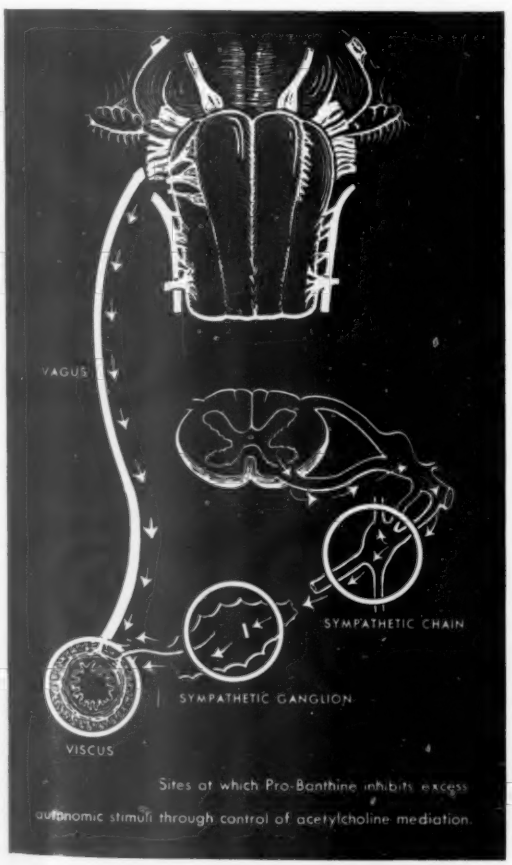

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1. Schwartz I. R.; Lehman, E.; Ostrove, R., and Seibel, J. M.: Gastroenterology *25*:416 (Nov.) 1953.

2. Roback, R. A., and Beal, J. M.: Gastroenterology *25*:24 (Sept.) 1953.

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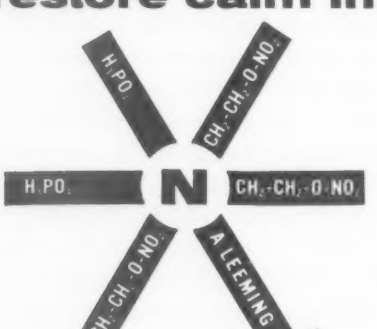

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ORIGINAL ARTICLES

THE PROBLEM OF THE ACTION OF INSULIN*

By William C. Stadie, M.D.
PROFESSOR OF RESEARCH MEDICINE

(From the John Herr Musser Department of Research Medicine, University of Pennsylvania, Philadelphia, Pennsylvania)

Today there are, in medical research, many fields of activity which have captured the imagination and aroused the fervor of scientists all over the world. Insatiable intellectual curiosity is, of course, the great driving force for these explorations. But each field has its special attractions. Why is it, one might ask, that diabetes mellitus has fastened its grip upon the imagination of physiologists, biochemists, and physicians for the past century or so? The answer, I believe, consists in this: That the manifestations of the disease cut across the whole range of metabolic activity in the mammalian organism. Not only is the metabolism of glucose distorted but the metabolism of proteins and of fats is perverted so profoundly that the very existence of the individual is threatened. But more than this, as we know, diabetes mellitus not only involves the pancreatic hormone, insulin, but also hormones of the pituitary and the adrenal gland. Thus the challenge of intellectual exploration is to the complex interplay of hormones with enzyme systems: an interplay which determines the chemical mechanisms by which our foodstuffs yield their energy for work, for growth, for regeneration of new tissue. It is no wonder that diabetes continues to be a subject of research in many laboratories throughout the world from which pour a continuous stream of literature which defies the devotion of any one person to master. To paraphrase Voltaire, one might say that if God had not created diabetes mellitus, man would have invented it . . . as indeed he has. For the affliction upon mankind of this malady has accelerated immeasurably the rate at which physiologists and biochemists have unravelled the complexities of metabolic activity within tissues.

The problem of insulin action began in 1890, with the familiar experiments of von Mehring and Minkowski. These investigators were not studying diabetes mellitus but functions of the digestive system. They removed the pancreas in the dog and, to their astonishment,

*The 1954 Mellon Lecture presented before the Society for Biological Research of the School of Medicine, University of Pittsburgh.

found they had produced a state of affairs closely resembling diabetes mellitus in the human. They deduced that the lack of an endocrine principle generated by the pancreas was responsible for the phenomenon.

This discovery stimulated much research related to the problem of insulin action. The controversy, now long forgotten, over the histological make-up of the pancreas ended when it was shown that the pancreas consisted of two independent organs totally unrelated in function: The acinar tissue and the islands of Langerhans. The islands survived blocking the pancreatic duct, but the acinar tissue atrophied. Removal of the island remnant was immediately followed by diabetes. Obviously, the endocrine factor assumed by Minkowski was produced by the islands.

But the isolation of this principle eluded the efforts of many trained research workers for many years until it was finally achieved by two who were relatively inexperienced in methods of physiological research. In 1922, Banting and Best prepared extracts which lowered the blood sugar from the remnants of the ligated pancreas.

The early work with insulin was chiefly concerned with answering the question as to what insulin did, rather than how it did it. Experimental work was done almost exclusively with the intact animal or, in some instances, perfused limbs or organs. The metabolist was concerned with what may be called "total metabolism." He measured body stores of carbohydrates, fat and proteins and how they were influenced by the disease or the administration of insulin. Much reliance was placed upon determination of respiratory quotients and of shifts of carbohydrate stores from one part of the body to another. From this kind of data two theories became formulated, each of which was defended with considerable warmth by their respective protagonists. These contending theories were summarized in the slogans: "Underutilization" and "Overproduction." The first theory contended that insulin was necessary for the combustion of sugar by the tissues. Without insulin the body was entirely dependent upon protein and fat. These metabolisms in turn became markedly distorted. The end result was a partial inability to use protein so that it became wasted excessively. An almost complete dependence upon fat as a source of energy resulted. But, unfortunately, in the absence of glucose oxidation, fat metabolism resulted in an unburnable residue of acid derivatives and, in consequence, a fatal termination in diabetic ketosis.

The opposing "Overproduction" hypothesis held that there was no impairment of glucose oxidation in diabetes. On the contrary, the function of insulin was to control the *production* of glucose from protein and fat. In the normal animal, insulin regulates with great nicety the metabolism of protein and fat so that just enough is metabolized to meet the energy demands of the body. In the absence of insulin, these processes were uncontrolled; in consequence, excess of both protein and fat metabolism produced sugar far exceeding the needs of the body and it was wasted in the urine. But, in addition, a side reaction of this excessive useless fat metabolism caused the formation of large amounts of ketone bodies and, as before, a fatal acidosis resulted.

But rapidly developing events soon showed that these concepts were oversimplifications of a very complex situation. Although the picture of insulin action at this time was quite confused, two things were obvious: 1) that diabetes mellitus could not be described solely in terms of a disturbed carbohy-

drate metabolism; 2) that it was difficult to ascribe to the pancreas alone the etiology of the disease. In many instances, the pathologist could not find enough evidence of island destruction to account for the severity of the disease. The clinician, likewise, had long suspected that endocrines other than insulin were involved.

The demonstration that insulin did not work in a hormonal vacuum came from an unexpected source. The Argentinian physiologist, Houssay[12], reported experiments on depancreatized animals from which the hypophysis had previously been removed. In these doubly operated animals, there is no diabetic syndrome. The hypophysectomized-depancreatized animal, to be sure, is in an unstable metabolic state and unable to withstand any stress; nevertheless, it shows a relatively normal type of carbohydrate, fat, and protein metabolism. This so-called Houssay phenomenon, which won its discoverer the Nobel Prize, has been demonstrated in many species of animals. It can also be demonstrated *in vitro* by the use of isolated surviving tissue from similarly operated animals. This is strikingly shown by illustrated experiments by Gurin and his group[2] on the synthesis of fatty acids by liver slices of depancreatectimized and Houssay cat and by work from our laboratory[29] on ketone formation by isolated liver slices from similar cats.

The first column of Table 1 shows the data of Gurin's group who measured the incorporation of isotopic acetate into long-chain fatty acids by liver slices *in vitro*. Compared to the normal, the depancreatized cat liver slices are deficient in fatty acid synthesis. But in the doubly operated Houssay animal, the fatty acid synthesized ability is restored to normal. In the second column of the table are the data from our laboratory showing ketone production by cat liver slices *in vitro*. In the case of the diabetic cat, the normal fatty acid metabolism with little or no ketone formation is replaced by the fatty acid metabolism of the liver characteristic of the diabetic, namely, high ketone formation. In the Houssay cat, the ketone production falls to low values, indicating a restoration of fatty acid metabolism toward the normal pattern.

TABLE 1.—DEMONSTRATION OF THE "HOUSSAY PHENOMENON" *IN VITRO* BY EXPERIMENTS WITH TISSUE PREPARATIONS FROM NORMAL AND DEPANCREATIZED CATS

	From Brady, Lukens, and Gurin[2] *Isotopic Acetate Incorporated by Rat Liver Slices into Long Chain Fatty Acids.*	*From Stadie, Zapp, and Lukens*[29] *Ketone Formation by Cat Liver Slices.*
	Mg./100 mg. Mean	*Micromoles/kg. Cat Mean*
Normal Cats	2–8	240
Depancreatized Cats	0–0.7	1200
Houssay Cats	5	85

Long and Lukens[16] later showed that the simultaneous removal of the adrenal in the depancreatized diabetic animal brought about an amelioration of the diabetes quite analagous to that following hypophysectomy. These observations generated the concept that

in the mammalian organism, in the absence of these three endocrine principles, there is a sort of primitive or fundamental metabolism ill-suited to great activity or stress. The superposition of balanced hormonal control endows it with versatility and range. The absence or excess of one or another hormone results in imbalance followed by abnormal types of tissue metabolism. These phenomena form the framework about which a new chapter in biochemistry is being constructed, namely, the interaction of hormones with each other and the tissue enzymes.

Meanwhile, the parallel development of enzymology began to have marked influence in directing research in the problem of insulin action. Beginning with the early work on fermentation by Pasteur and others, enzymology grew into a complex science by means of which many of the metabolic reactions occurring in living mammalian tissues could be explained. Many of the enzymes, with their amazing ability to catalyze complex reactions with smoothness and speed far beyond the capacities of the organic chemist, were separated from tissues and crystallized and the coenzymes necessary for their action isolated and purified. At present, it is possible to say that almost all of the reactions involving glucose from its absorption to its final disposition as CO_2 and water are known with considerable precision. In the similar fields of protein and fat metabolism, an excellent beginning has been made, but much uncertainty still exists.

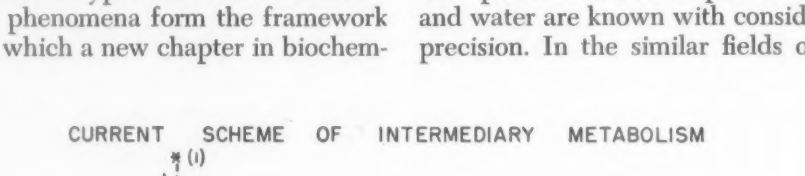

Fig. 1.—Current scheme of intermediary metabolism. The possible metabolic defects in diabetes are shown by: *1—transfer; *2—hexokinase; *3—oxidative phosphorylation; *4—Krebs cycle.

The enzymatically catalyzed reactions, by which glucose is prepared for oxidation or incorporation into synthetic reactions, comprise the glycolytic cycle and this has become the map by which the metabolist studying hormonal inter-relations steers his experimental course. This glycolytic cycle is shown in Fig. 1.

It is universally agreed that glucose

(as well as other sugars) must be converted to hexosephosphate as the initial step in mammalian intermediary metabolism. In the case of glucose, glucose-6-phosphate results from the reaction of glucose with adenosine triphosphate. The reaction is catalyzed by the enzyme, gluco-hexokinase, a ubiquitous enzyme present in all tissues. This reaction is irreversible. From glucose-6-phosphate the paths diverge: (1) to glycogen by a series of catalyzed reactions; or (2) after a second phosphorylation, the phosphorylated hexose is split to form two molecules of triose phosphate. One oxidation step occurs in the glycolytic cycle (indicated in the schema by the removal of 2 hydrogens). Accordingly, each triose molecule is oxidized to pyruvic acid. The entire function of the glycolytic cycle can be summed up by saying that it converts glucose into pyruvic acid. All the reactions in the glycolytic cycle proper are reversible so that glycogen or glucose phosphate may go to pyruvic acid or *vice versa*.

A pathway of glucose metabolism other than through the glycolytic cycle to pyruvate has recently been much emphasized. Glucose-6-phosphate is oxidized to phosphogluconate, a reaction catalyzed by Warburg's Zwischenferment. Further reactions along this pathway occur leading to complete oxidation to CO_2 and water. According to Stetten, about half of glucose oxidation in the rat liver goes by this route, but little, if any in muscle. Nothing is known about hormonal control of this system, if such exists, and hence we shall dismiss it from further consideration.

A second development of prime importance to the tissue metabolist was the demonstration of a cyclical oxidative system in tissues whose function is to engage the end product of the glycolytic cycle, namely pyruvic acid, in a chain of reactions leading ultimately to the terminal production of CO_2 and water. This Krebs or citric acid cycle (shown in Fig. 1) is linked to the glycolytic cycle by a reaction in which the three carbon pyruvic acids condense with the four carbon oxalacetic acids to form the six carbon citric acid; one carbon is eliminated as CO_2 in this step.

Figure 1 also shows that the initial condensation of pyruvic acid with oxalacetic acid is followed by three separate steps of oxidative decarboxylations, and the original oxalacetic acid is regenerated by two further oxidation steps to begin the cycle anew. One cycle thus results in the complete oxidation of pyruvic acid to CO_2 and H_2O.

A further development in enzyme chemistry remains to be considered. Within the mitochondria of all tissue are situated all of the enzymes and coenzymes concerned in the Krebs cycle. The innumerable oxidative reactions which occur upon introduction of metabolites derived from glucose, fat, or protein into the Krebs cycle result in the formation of high energy phosphate bonds which are stored as adenosine triphosphate or creatine phosphate. But, by an extraordinary mechanism for efficient conservation of energy, a single oxidative step of a metabolite involving the removal of two hydrogens generates a succession of phosphate bonds as the hydrogen pair is passed along a series of enzymatic or coenzymatic electron carriers. As many as four energy rich phosphate bonds may thus be generated per atom of oxygen used. This P:O ratio, as it is called, measures the efficiency of the system. The efficiency of the total reactions is maintained at a very high level, actually 70 to 90% of the original energy of the foodstuff being stored as adenosine triphosphate or creatine phosphate. These compounds, created by special-

ized reactions of phosphorylation, endow the tissue with an ever-present supply of chemical energy for the innumerable energy-requiring reactions of the cell such as synthesis, mechanical and osmotic work, secretion, and so forth. The striking developments in the biochemistry of phosphates in recent years has raised it to the rank of an independent chapter claiming the devotion of many scientists and the accumulation of a literature of great importance. It was inevitable that possible failure to function of a system of this importance should be suspect as an etiological factor in diabetes.

Of equal importance to these new developments in theory was the great advance in methodology due to the ready availability of compounds labelled with radioactive and other isotopes. By their use, it is possible to follow the course of isotopic metabolites through the maze of chemical reactions within the cell and answer questions which otherwise would be completely impossible to answer. A striking example of this is illustrated in the old controversy as to whether or not insulin increased the immediate oxidation of glucose. As recently as 1946, Soskin and Levine[25] stated: "One of the most intrenched notions about insulin . . . is that it increases the dissimilation* of carbohydrate. This is without basis in fact . . ." Wick, Drury, Bancroft and MacKay[32] performed the simple experiment of injecting isotopic glucose into eviscerated rabbits and by determination of the radioactivity of the expired CO_2 calculated the glucose oxidized under the influence of insulin. Part of their data are shown in Table 2. Their conclusion is simple: "Our results give positive proof that insulin increased the combustion of glucose by the extrahepatic tissue."

This preliminary discussion of some fundamental considerations of intermediary metabolism is necessary, I believe, for an adequate understanding of the problem of the action of insulin. The map which will guide us is contained in the schema of intermediary metabolism which gives the inter-relation of the glycolytic and Krebs cycle (Fig. 1).

TABLE 2.—OXIDATION OF U–C^{14} GLUCOSE BY EVISCERATED RABBITS: CALCULATED FROM EXPIRED CO_2 (Wick, Drury, Bancroft, and MacKay)[32]

	No Insulin	*Insulin*
Glucose Oxidized, mg.	543	2144

Glucose, before it can come into the range of the action of enzymes within the cell, must pass the barrier of various cytostructural interfaces. This is shown in position I on the chart. The modern view of the cell is not to regard it as similar to a collodion sac with a more or less structureless membrane containing a mixture of enzymes in simple solution. On the contrary, there are subcellular structures containing systems of enzymes separated from each other by interfaces which may have individualized properties of permeability. Examples of such sub-cellular structures are: nuclei, mitochondria, microsomes. It is possible that these interfaces, as well as the cell membrane proper, may impose barriers to the ready entrance of glucose or other metabolites and that these impeding forces may be increased or decreased by hormonal action. There is, therefore, the first possibility that the permeation of glucose into the cell may be under control of insulin.

A second possibility of insulin action is its acceleration of the conversion of glucose into an "active" form. This

*By dissimilation Soskin and Levine mean the complete breakdown of a substrate to CO_2 and H_2O[25], p. 148.

idea that glucose must be "activated" before it is susceptible of metabolism is an old one and many forms of glucose have been proposed as active forms. But it is now generally accepted that all mammalian glucose metabolism is proceeded by a phosphorylation to glucose-6-phosphate, formed by the action between glucose and adenosine triphosphate catalyzed by the enzyme gluco-hexokinase (indicated by 2 in Fig. 1). This enzymatic system is a second possible site of insulin reaction.

A third site of insulin action may be on the many enzyme-coenzyme systems in the Krebs cycle which are concerned with the oxidative generation of energy-rich phosphate bonds. It is easy to understand that a deficiency of the supply of energy-rich phosphate bonds through insulin lack might cause disturbances in carbohydrate metabolism through lack of ATP for glucose activation. Furthermore, the metabolism of fat and protein are also dependent on supplies of energy-rich phosphate bonds; hence these metabolisms may also be disturbed by the same mechanism. It is also conceivable that permeation of glucose into cells may require energy-rich phosphate.

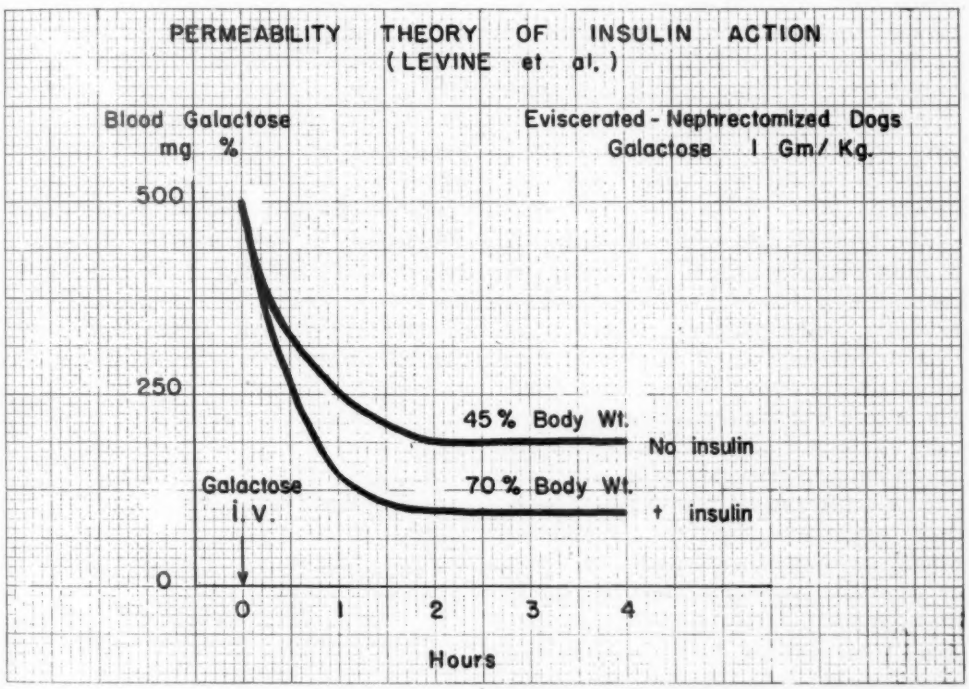

Fig. 2.—Acceleration by insulin of the transfer of galactose across cell barriers in eviscerated-nephrectomized dogs: Galactose± insulin was injected at 0 hours; galactose space calculated from blood galactose. Insulin increased galactose space from 45% to 70% of body weight. Schematized from data of Goldstein, Huddlestun, and Levine[8].

I have thus named three possible types of insulin action: 1) on permeation, 2) on activation, and 3) on oxidative phosphorylation. There is a possible fourth, namely, on oxidative reactions in the Krebs cycle. Krebs, himself[14], was first to point out a possible

effect of insulin in the Krebs cycle in pigeon breast muscle. But this phenomenon, which was abundantly confirmed by others, has been somewhat neglected in the recent literature, and I shall omit further reference to it.

The concept that permeability of cell membranes or subcellular structures is influenced by hormones is not a new one. But the first convincing evidence that permeation of sugars was affected by insulin was presented by Levine and his co-workers[15]. They injected a sugar into the experimental animal and, from the blood sugar levels after equilibrium, calculated the body space in which the sugar was distributed.

The type of data which he obtained is illustrated in Fig. 2. Levine *et al.* used eviscerated dogs injected intravenously with galactose. There were reasons for this: 1) It is necessary to eliminate the liver, kidney, and intestine to prevent the conversion of galactose to glucose. 2) It is necessary to use a sugar like galactose which is metabolically inert in muscle. Under these circumstances the "galactose space" becomes a measure of the permeation of sugar into the muscle. In the controls, Levine found the galactose space was 45% of body weight. But when insulin was administered simultaneously with the galactose to the eviscerated preparation there was an increase of galactose space to approximately 80%, which represents the space occupied by body water. Similar experiments were done by the authors using sugars other than galactose. Levine concluded as follows: "There exists a barrier on the surface of certain cells which opposes the entrance of sugars and insulin facilitates a transfer system capable of carrying sugars of a given chemical structure into the interior of the cell. The metabolic effects of insulin would thus be secondary to a more rapid rate of entry of the sugar into the cell."

Levine and his co-workers[8] reported similar studies on a variety of sugars. These, they divided into "insulin responsive" and "insulin nonresponsive." Those which were insulin responsive were characterized by having similar configurations in the first three carbon atoms. But this latter conclusion must be questioned, since exceptions to Levine's findings have been found by others. For example, fructose is a ketose, not an aldose like glucose; but, its metabolism by the rat diaphragm is influenced by insulin. From their observations, however, the authors concluded that the entrance of the sugar into tissues is "governed by a transfer system which is especially adapted with respect to chemical structure." This transfer system whose mechanism at present is obscure is under hormonal control.

TABLE 3.—"FREE" GLUCOSE IN HEART, SKELETAL MUSCLE, AND DIAPHRAGM OF RATS FOLLOWING INJECTION OF ISOTOPIC GLUCOSE.

(Schematized from data of Park)[19]

	"Free" Glucose in Tissues
No Insulin	Extra-cellular distribution
With Insulin	Distribution increased 4–5 fold

Experiments supporting these conclusions of Levine were reported by Park[19]. The data shown in Table 3 were obtained from this report. Park used a different approach. He measured the rate of accumulation of free glucose in the intracellular space of muscle. Ordinarily, glucose entering cells is rapidly phosphorylated so that no "free glucose" accumulates intracellularly. Park circumvented this difficulty by using isolated rat diaphragms in chilled medium, thus slowing the reaction of phosphorylation. The glucose within

the cell which had thus escaped combination with phosphate, that is, "free glucose", was determined by appropriate methods. Park's data show: 1) "The quantity of 'free' glucose recovered from the non-insulinized tissue approaches closely the expected value for an exclusively extra-cellular distribution at all serum glucose levels between 300 and 1000 mg. per 100 cc. 2) In insulinized animals, however, the quantity of free glucose found in the heart or the diaphragm was 4 to 5 times greater than in non-insulinized tissues." Results with skeletal muscles were in the same direction but more irregular, presumably due to relatively poor circulation. Park's conclusions were 1) without insulin, the permeation of glucose into the cell is so slow that the hexokinase reaction immediately converts it into glucose-6-phosphate so that no "free" intra-cellular glucose accumulates; 2) with insulin, the rate of entrance is greatly enhanced and exceeds the rate of phosphorylation. Hence, intracellular "free" glucose accumulates.

Mirsky and his co-workers[9] also studied rates of penetration of sugars into the rat diaphragm *in vitro*. Their conclusions were limited to sugars which were metabolically inert and in the case of d-galactose, for example, they reported that insulin accelerated intra-cellular transfer.

Further confirmatory evidence that insulin is concerned with glucose permeation was published by Ross[21,22] of the London Ophthalmological Institute. He used the ciliary body of the rabbit, which is known to oppose a barrier to permeation of blood glucose into the aqueous humor of the eye, as a system for testing the effect of insulin upon permeation. It is possible in the rabbit to vary the blood sugar level at will and draw samples of aqueous humor from time to time from the anterior chamber for glucose analysis. Using such methods, Ross obtained kinetic data and expressed his results in terms of a velocity constant k_{in} which is a measure of rate of transfer of glucose across the ciliary body into the anterior chamber of the eye. Representative data are shown in Table 4.

TABLE 4.—TRANSFER OF GLUCOSE ACROSS THE BLOOD-AQUEOUS BARRIER IN THE EYE OF NORMAL AND ALLOXANIZED RABBITS:
(Ross[21,22])

	1000 k_{in}
Normal	199±36
Alloxanized (Blood glucose 400 to 500 mg. per 100 cc.)	102±10
Decrease	97±38
Fisher's test for significance	t=2.5

In the normal, what Ross calls the diffusion constant inward, was about 200 and in the alloxanized, with an elevated blood sugar of 400 to 500 mg. per 100 cc., the value is significantly lower. All of the rather extensive data published by Ross using this system lead to the same conclusion, namely, that the transfer of glucose across a cell barrier in the eye is influenced by insulin.

These experiments indicate that cellular interfaces impose a barrier upon the ready entrance of glucose and other sugars across them. This barrier may be lifted by the action of insulin, but the manner by which this is achieved is at present unknown. There is hesitancy in attributing this phenomenon to alterations in permeability due to some physical mechanism. At present, it is better to call it simply "glucose transfer" thus avoiding all commitments as to possible mechanisms.

The entrance of glucose into the cell must be followed by activation be-

fore further catabolism occurs. It is universally agreed that glucose activation is accomplished by the formation of hexose-6-phosphates by reaction with ATP catalyzed by the enzyme glucose-hexokinase. Early reports[6a] indicated that the hexokinase reaction in homogeneous extracts from muscle of normal and diabetic rats is inhibited by pituitary and adrenal preparations and that this inhibition was released by the addition of insulin. Reports from many laboratories failed to confirm these observations, and we can say that there is no unequivocal proof available that insulin or pituitary or adrenal, singly or together, have any influence upon the hexokinase reaction *in preparations which have cytological structures destroyed.*

At present, we are forced to believe that hormonal interaction upon enzyme systems is dependent upon intact morphology, although to what degree preservation of structure is necessary is not known. But dogmatization is hazardous, for we must remember that Pasteur, in 1860, said: "The chemical act of fermentation is essentially a phenomenon correlative with a vital act commencing and ceasing with the latter. I believe that alcoholic fermentation never occurs without simultaneous organization, development, and multiplication of cells, or the continued life of cells already formed." This view was shown to be wrong by Buchner's demonstration that cell-free yeast extracts cause fermentation of glucose to alcohol and CO_2.

The failure to show by experiments on cell-free preparations that the gluco-hexokinase reaction was involved in diabetes necessitated devising experiments using morphologically intact tissue to study the possible existence of such a defect. This could be accomplished by making use of two established phenomena.

1. Tissues contain hexokinases specific for certain sugars. Thus liver has both a gluco-hexokinase and a fructo-hexokinase whose actions are completely independent one of the other. The first method, then, for studying the hexokinase reaction in intact tissue would be by a comparison of the metabolism of the two sugars in normal or disturbed endocrine states.
2. The second method is based on the possibility of studying the effect of insulin on metabolic reactions of glucose beyond the initial phosphorylation step. These reactions involve members of the glycolytic cycle which are reversibly convertible into each other. Furthermore, the reactions concerned are believed to be independent of hormonal control. Hence, comparison in various endocrine states of the metabolism of members of the glycolytic cycle with that of glucose should be informative about the one reaction, the hexokinase reaction, which is not common to the two processes.

I shall discuss two reported experiments of the first type. Cori and Cori[6b] injected eviscerated rats with two sugars: glucose and fructose with or without insulin.

TABLE 5.—INFLUENCE OF INSULIN ON THE UTILIZATION OF GLUCOSE OR FRUCTOSE BY EVISCERATED RATS:

(Cori and Cori)[6b]

	% Sugar Recovered After 60 Min.	
Sugar Injected	*No Insulin*	*With Insulin*
Glucose	90	2
Fructose	61	62

At the end of 60 minutes, the carcass was analyzed for residual sugar and the results shown in Table 5 were obtained. The data show, in the case of fructose, that insulin was without effect but that the disappearance of glucose was greatly accelerated. The authors concluded that the kinases concerned in the activation of these sugars were different. That for glucose is accelerated by insulin; that for fructose is not.

An experiment of similar type has recently been reported by Renold, Hastings and Nesbett[20] (1954). The metabolism of isotopic glucose and fructose by liver slices from normal and diabetic rats was studied and the data given in Table 6 was obtained.

TABLE 6.—COMPARISON OF THE METABOLISM OF GLUCOSE AND FRUCTOSE BY LIVER SLICES FROM NORMAL AND DIABETIC RATS:

(Renold, Hastings, and Nesbett)[20]

	Micromoles per gm. in 90 min.	
Total Uptake:	*Normal*	*Diabetic*
Glucose	37	14
Fructose	96	99
Glycogen:		
From Glucose	15	0.3
From Fructose	18	2.5

The uptake of glucose by liver slices from the diabetic rat was reduced from 37 to 14; that of fructose was unchanged. The authors concluded from these and other data that "whereas liver slices from normal rats convert approximately equal amounts of glucose and fructose to glucose-6-phosphate, phosphorylation of glucose by liver slices of diabetic animals is but one-tenth to one-fourth that of fructose." It is worthwhile pointing out, however, that these authors also found that the synthesis of glycogen from fructose as well as glucose is impaired in the diabetic rat liver. This must mean that a defect other than the initial phosphorylation of glucose or fructose is involved in the diabetic state.

Experiments of the second type, namely, comparison of the metabolism of glucose with that of members of the glycolytic cycle, are illustrated by some data also from Hastings' group.[31].

The experimental data show the synthesis of glycogen by rat diaphragm equilibrated *in vitro* with isotopic glucose, glucose plus pyruvate, or pyruvate. Three types of experimental rats were used: The normal, the adrenalectomized, and the diabetic adrenalectomized. The authors state that in the diabetic-adrenalectomized rat the inhibitory action of the pituitary upon the gluco-hexokinase reaction is unopposed. Accordingly, there should be a marked decrease in glycogen synthesis from glucose, since this reaction requires hexokinase which presumably is inhibited. On the other hand, in the formation of glycogen from pyruvate by reversal of the glycolytic cycle, gluco-hexokinase reaction is not involved, so that no decrease of glycogen synthesis from pyruvate should be observed. And

TABLE 7.—INCORPORATION OF ISOTOPIC GLUCOSE OR PYRUVATE INTO GLYCOGEN BY SURVIVING DIAPHRAGMS FROM NORMAL OR DIABETIC-ADRENALECTOMIZED RATS:

(Data taken from Teng, Sinex, Deane, and Hastings)[31]

	Glycogen Synthesis mg./100 mg.	
	From Glucose	*From Pyruvate*
Normal	120	100
Diabetic-adrenalectomized	40	140

as the data in Table 7 show, this was observed in fact. When both glucose and pyruvate were in the medium, there is also no significant decrease of glycogen synthesis in the diabetic adrenalectomized preparation. These experiments and others of the same type reported in the literature led the authors to conclude that the hexokinase reaction in intact tissues was inhibited by lack of insulin and accelerated by its presence. Can we now accept these conclusions? The answer, I believe, is: "With reserve" for the strong evidence reported by Levine, by Park,

by Ross and by Mirsky shows that insulin is concerned with a "transfer mechanism" accelerating the entrance into the cell of certain sugars and not of others. This makes it difficult, if not impossible, to decide whether the acceleration of reactions involving glucose by insulin is due to its influence on the hexokinase reaction or on the acceleration of glucose transfer across cell boundaries. I know of no experiments yet devised which will make the choice between these hypotheses possible, except the demonstration of insulin action upon hexokinase in homogeneous systems; and this, as we have seen, has not been accomplished. We thus are faced with a dilemma which, at present, is insoluble.

Turning back to the schema of intermediatry metabolism of glucose (Fig. 1), it is to be noted that the oxidative reactions in the Krebs cycle are concerned with the generation of high energy phosphate bonds which are stored in the form of ATP and creating phosphate. The formation of glucose-6-phosphate, many synthetic reactions including the synthesis of fatty acids and protein, and perhaps glucose transfer are dependent upon supplies of these energy-rich phosphate bonds. There is much evidence in the literature indicating possible involvement of insulin in energy-rich phosphate formation. A few representative types of data are shown.

TABLE 8.—PHOSPHORYLATION BY RAT DIAPHRAGM EQUILIBRATED WITH GLUCOSE N = 8:

(Haugaard, Marsh, and Stadie)[11]

	Insulin Effect $\mu M/gram/hr.$
Δ Ester P	4.6 ± 1.19
Δ Acid-labile P (ATP)	-3.0 ± 1.16
Δ Glycogen (Glucose equiv.)	10.6 ± 0.90
Sum	12.2 ± 1.9

The data in Table 8 are from experiments in our laboratory[11]. Diaphragms from normal rats were equilibrated *in vitro* in a glucose medium with and without insulin. The effect of insulin upon oxidative phosphorylation was calculated as the sum of its effect on the phosphate esters of glucose and adenosine triphosphate. To these is added the insulin effect on glycogen synthesis which requires one stage of phosphorylation. The sum of these represents the minimum value for the effect of insulin on the phosphorylation of the glucose. The data show this effect of insulin was to increase phosphorylation by 12 micromoles per gm. of tissue per hour. The point to be emphasized is that this extra phosphorylation was not accompanied by an increase of oxygen uptake or R.Q. The possibility that the efficiency of the coupling between oxidation and phosphorylation may be dependent upon the endocrine state of the tissue is a real one. For this reason, we interpret our experiments to indicate that insulin has increased significantly the efficiency of oxidative phosphorylation in the rat diaphragm, since the extra energy-rich phosphate bond production was not accompanied by extra oxygen consumption.

Much further evidence along this line might be quoted. For example, the turnover of ATP and hexose phosphates is accelerated in liver and muscle by injection of insulin into the intact animal. The phosphorylation of hepatic thiamine has been shown to be impaired in the diabetic animal and restored by insulin. (Siliprandi and Siliprandi[24]).

An interesting study on phosphorylation is summarized in Fig. 3. Charalampous and Hegsted[3] studied the rate of acetylation of a foreign amine, namely, para-aminobenzoic acid, when injected into normal and diabetic rats. This

acetylation is dependent upon the availability of energy-rich phosphate bonds. The data in Fig. 3 show that acetylation as calculated from the urinary excretion of the acetylated para-aminobenzoic acid is significantly diminished in the diabetic animal. The authors state that "it is reasonable to conclude that a primary deficiency of adenosine triphosphate synthesis is the cause of the metabolic effect.

We have recently obtained some direct evidence of possible defective oxidative phosphorylation in the diabetic state[28]. Oxidative phosphorylation is believed to occur exclusively in the mitochondria. We have, therefore, compared the phosphorylative ability of liver mitochondria from normal and depancreatized cats using two nutrilites, namely, pyruvate and beta oxybutyrate, as sources of energy. The data showing ATP synthesis by mitochondria from normal and depancreatized cats are shown in Table 9. The mitochondria from depancreatized cats,

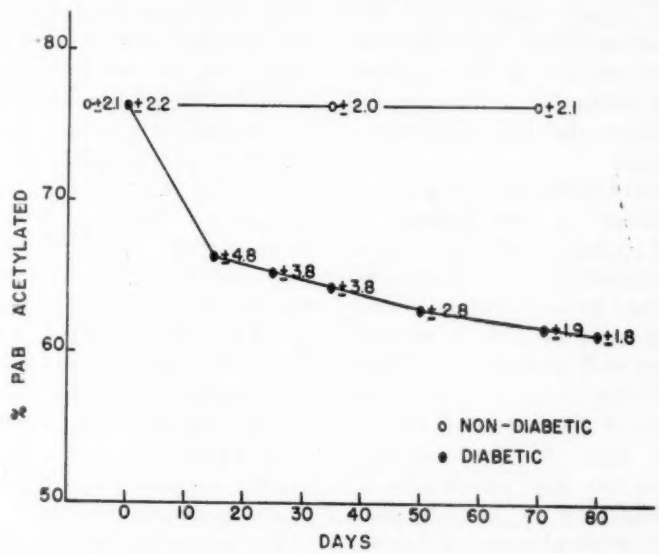

Fig. 3.—Comparison of acetylation of para-aminobenzoic in normal and alloxanized rats. Note the sharp decrease about 10 days after alloxanization. (The figures on the graph are the standard deviations of each group: Charalampous and Hegsted[3].)

TABLE 9.—OXIDATIVE PHOSPHORYLATION BY LIVER MITCHONDRIA PREPARED FROM LIVERS OF NORMAL AND DEPANCREATIZED CATS:

Data give rates of formation of adenosine triphosphate (μM per minute per mg. of mitochondrial protein) in presence of pyruvate or beta hydroxybutyrate.

(Stadie and Vester)[28]

	Pyruvate		*β-hydroxybutyrate*	
Cat No.	*Norm.*	*Diab.*	*Norm.*	*Diab.*
1	60	7	31	15
2	71	18	42	34
3	52	13	30	5
M	61	13	34	18

compared to those prepared from normal cats, showed significantly lower synthesis of ATP with both substrates. The P:O ratio, which is a measure of the efficiency of the phosphorylative systems, was also lower in the diabetic cats. These preliminary experiments appear to give direct evidence for the impairment of oxidative phosphorylation by mitochondria in the diabetic animal. Corroborative evidence is needed to define the full significance of these findings.

The relation of these three postulated metabolic defects in the diabetic to the disturbed carbohydrate metabolism are easy to understand, but their relation to disturbed fat and protein metabolism is more complex and, at present, far from being well understood. A brief discussion of this area is appropriate in a discussion of the action of insulin.

Ten years ago, the intermediary metabolism of fat was essentially an uncharted field but is now rapidly developing. Newfound metabolic accessory activators, such as coenzyme A, ATP, and "active acetate," have been shown to have essential roles in the synthesis and oxidation of fatty acids. The effect of hormonal interaction upon these complex reactions is just beginning to be understood.

It has long been known that fat metabolism is disturbed in diabetes. Drury[7] was the first to show by unequivocal experiments that insulin accelerates fat synthesis. Stetten and Boxer[30] first quantitated the extent of this defect in the diabetic rat by studying the rate of incorporation of deuterium into the body fat of normal and alloxanized rats. They calculated that, compared to the normal, the "utilization of glucose for fatty acid synthesis is reduced to about 5%" in the diabetic rat. Further reports appeared in the literature indicating that[1,4] products of glycolysis were necessary for synthesis of fatty acids. The failure of this synthesis in the diabetic appeared to be secondary to the inability to bring about glycolysis of glucose. For example, Chaikoff and his group[1,4] placed diabetic rats on a 5 to 6 day regimen of prior feeding with glucose or fructose, then measured fat synthesis of surviving liver slices by measuring the incorporation of isotopic acetate into higher fatty acids.

Antecedent glucose feeding had no effect on the inability of the slices to synthesize higher fatty acids, whereas antecedent fructose feeding restored it to normal. Chaikoff concluded that one diabetic effect occurred early in the glycolytic scheme, namely, at the gluco-hexokinase level. On the other hand, the hexokinase specific for fructose, fructo-hexokinase, was unaffected by the diabetic state. Hence, glycolysis of fructose was unimpaired in consequence of which fat synthesis was restored to normal levels.

The necessity for the active metabolism of glycolytic products as a requirement for fatty acid synthesis is shown by experiments from our laboratory showing the relation between the initial glycogen content of livers from rats and the ability of slices from these livers to incorporate isotopic acetate into fatty acids[10].

We show here a series of liver slices from rats in which the initial glycogen content was made to vary by antecedent periods of starvation which were obtained from a series of normal rats. The ability of these slices to incorporate labelled acetate into higher fatty acids was measured, following a suitable period of equilibration *in vitro*. The data (Fig. 4) show that the higher the initial glycogen, the greater is the ability of the liver slices to synthesize fatty acids from the acetate. We interpreted this to mean that glycolytic products from glycogen must be avail-

able for the liver to achieve this synthesis. More direct proof of this hypothesis was reported by Shaw and Gurin[23].

They prepared a system which was entirely free of formed elements from the liver of depancreatized pigeons. This contained the enzymes capable of synthesizing fatty acids derived by lysing the mitochondria and also the soluble hepatic enzymes capable of glycolysis. A summary of their data is shown in Table 10. Such a preparation from normal pigeon livers readily incorporated isotopic acetate into the higher fatty acids. On the other hand, a similar preparation from depancreatized pigeon livers by itself had essentially lost this power. When metabolites capable of glycolysis, such as glycogen, glucose-6-phosphate, or fructose-1-6-phosphate

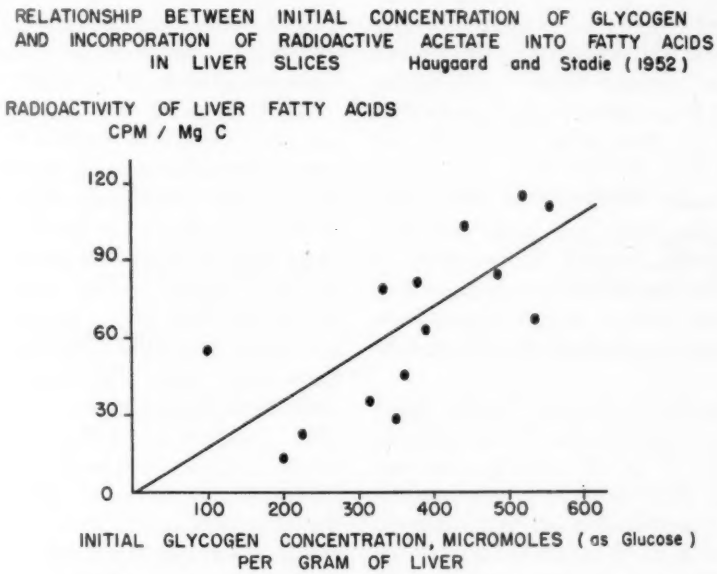

Fig. 4.—Relationship between initial concentration of glycogen and incorporation of radioactive acetate into fatty acids in liver slices: Haugaard and Stadie[10].

TABLE 10.—LIPOGENESIS BY PARTICLE-FREE EXTRACTS OF LIVERS FROM DEPANCREATIZED PIGEONS:

(Shaw and Gurin)[23]

Source of Enzyme System	*Incorporation of Isotopic Acetate in Fatty Acids. Micromoles/10 mg. Fatty Acids*
Normal Pigeons	0.61
Diabetic Pigeons	
Additions to Medium	
No additions	0.1
Glycogen	1.0
Fructose-1-6-diphosphate	1.7
Glucose-6-phosphate	0.3
Insulin	No effect

were added, higher fatty acid synthesis from acetate was restored as the data in the table show. We might say that a "diabetic" solution of enzymes was "cured" by the addition of a metabolite. It is to be noted that insulin had no effect whatever upon this system, reinforcing our previous comment that organization is a necessary requirement for insulin action. Shaw and Gurin concluded from these experiments that "lipogenesis is dependent upon efficient glycolysis."

Much more evidence of this type might be discussed but, in general, the evidence with respect to the role of insulin in fat metabolism indicates that the diabetic defect in fat metabolism is secondary to deficient glucose metabolism.

We turn for a moment to the effect of diabetes and insulin upon protein metabolism. It is, of course, common knowledge that there is a disturbance of protein metabolism in the diabetic characterized by a marked increase of nitrogen output, indicating an increased breakdown of protein. It has been well established that insulin accelerates the synthesis of protein. For example, Mirsky and others[18] have shown in a variety of animals that injected insulin accelerates the fall of blood amino acids presumably due to increased synthesis of body protein. Lotspeich[17] studied the rates of disappearance of ten amino acids from the blood of dogs treated with insulin and found a correlation between these rates and the proportion of each amino acid in skeletal muscle protein. This was interpreted to mean that insulin promotes protein synthesis. Krahl[13] recently has studied the incorporation by liver slices from the normal and diabetic rats of isotopic glycine into glutathione. His data show that this incorporation is diminished in the diabetic rat, but that the presence of insulin in the medium, particularly if glucose is also present, restores the glycine incorporation essentially to normal. Krahl concluded "that these experiments indicate that there is a direct dependence of glutathione synthesis in liver slices upon the availability of carbohydrate metabolic intermediaries." The situation with respect to the action of insulin upon protein synthesis is, therefore, the same as in the case of fat.

At this point we must pause and ask an important question: In what terms have we been describing insulin action? My answer is: We have been writing equations for the chemical reactions which occur when a metabolite is acted upon by an enzyme system which itself is being acted upon by a hormone. But this is not enough. We must ask: How are these effects accomplished? Surely, some sort of molecular mechanism must be involved which has not yet been formulated in chemical or physical terms. Contrast the situation in enzyme-substrate relations. Here the Michaelis-Menten concept[5], based on the assumption that there is a chemical combination between enzyme and substrate, has been firmly established and has proven to be of great usefulness. Certainly, in the case of insulin, the first step in its action must be its entrance into the cell and approach within molecular distance of the enzyme system which it effects. It would hardly be surprising that such a close approach would result in a firm association so that a chemical "binding" of insulin with reactive loci of the cell would exist. We first demonstrated this possibility by a simple experiment: A surviving hemi-diaphragm of a rat was dipped for 10 seconds in a medium containing glucose but no insulin. Invariably, these insulin pre-treated diaphragms synthesized extra amounts of glycogen compared to controls. Numerous experiments[26] indicated that the insulin was not "free" insulin in solution

and we concluded that the extra synthesis of glycogen was due to insulin bound to tissue sites in such a way that it exerted its customary action upon metabolic reactions. Metabolic activity of bound insulin has been demonstrated in rat diaphragm, lactating mammary tissue, and in adipose tissue. We also showed that the action of the bound insulin was altered by the endocrine state of the experimental animal, being diminished in alloxan diabetes, increased following hypophysectomy, and decreased by prior injection of pituitary and adrenal hormones.

The phenomenon of binding and the availability of labelled insulin made it possible for the first time to measure exactly the biological effect of a hormone on a specific metabolic process and express it in terms of units of weight[27].

We used the rat diaphragm and varied the amount of bound insulin by varying the duration of preliminary exposure to radioactive insulin. After washing, the diaphragms were equilibrated in glucose medium and the extra glycogen synthesis and bound isotopic insulin, measured. The data in Fig. 5 show that the insulin effect on glycogen synthesis increased as the bound isotopic insulin was increased. The slope of the line is the biological activity of a microgram of insulin bound to 1 gm. of tissue. The value obtained is 3.6 ± 0.76 mg./hr./μgm. of bound insulin by 1 gm. of tissue. Similar values were obtained if the isotopic insulin was introduced into the diaphragm by injection into the intact rat, diaphragms from uninjected rats being used as controls.

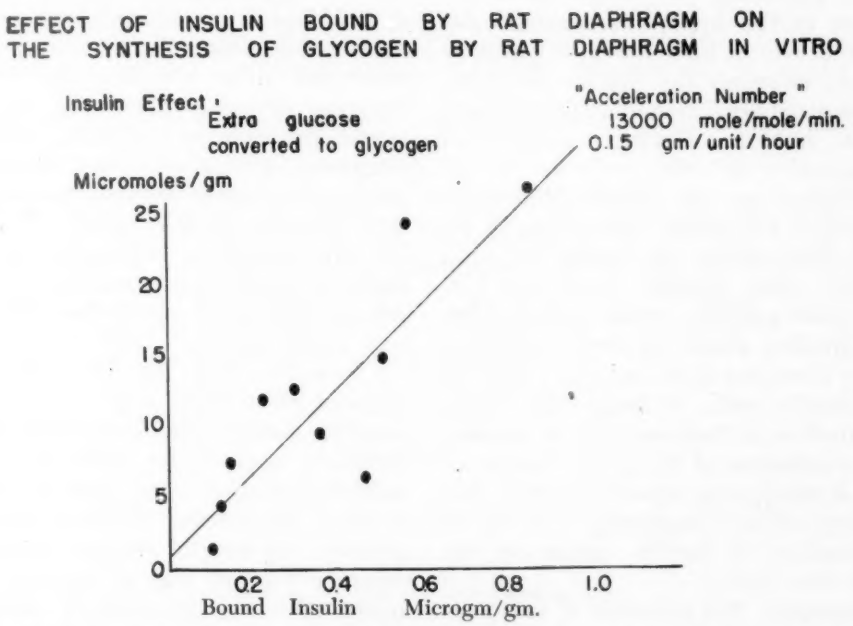

Fig. 5.—Relation between the insulin bound by diaphragm of normal rat and its effect on the synthesis *in vitro* of glycogen from glucose: Stadie, Haugaard and Vaughan[27].

Similar determinations of the biolog-

ical activity of bound insulin were done using diaphragms from hypophysectmized rats. The results clearly indicated that the activity of insulin in producing extra glycogen synthesis from glucose per microgram of bound insulin is strikingly increased in the hypophysectomized rats, particularly when the amount of bound insulin is small. Low bound insulin, which in normal diaphragms produce no increase of glycogen synthesis, give almost maximum effects in the hypophysectomized rat. In other words, the removal of pituitary factors increases the insulin reactivity of the peripheral tissue, a controversial point hitherto lacking unequivocal evidence.

Whether or not insulin binding is necessary for insulin action cannot be said. The ability of tissues to bind insulin varies greatly. Liver and kidney bind actively; white cells exhibit the greatest ability to bind; curiously, brain does not bind insulin, a fact in conformity with evidence that brain metabolism is independent of insulin. The evaluation of the phenomenon requires much more experimentation, but perhaps offers a beginning basis for an explanation of insulin action at the molecular level.

Summary. The advances of enzymology and the availability of isotopes, since the discovery of insulin, have brought about a complete change in our views of the diabetic defect. Then we had two poorly defined theories contending for supremacy. Now we have three theories much better understood but still contending. The first implicates barriers across cytological boundaries interfering with glucose transfer; the second, defects in glucose phosphorylation; and the third, diminished oxidative phosphorylation. But this is not all: There are well established observations of insulin action that do not fit readily into any of the above possibilities. To make the situation still more complex, the entire interplay of other hormones, for example, pituitary and adrenal, must be integrated into a complete theory of insulin action. It is obvious that we are far from such an integration and anyone who hazards an explanation of diabetes or insulin action on the basis of one mechanism is apt to find himself very much out on a limb.

I bring to a close this discussion of a segment of a great area of scientific inquiry; namely, the interaction of a hormone upon tissue enzymes. Will anything practical come from this type of study of diabetes mellitus? Such a question requires no answer. The physician will never rest so long as any aspect of vital phenomena remains obscure. Practical results are the inevitable fruit of this unconquerable drive for intellectual exploration.

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PSITTACOSIS IN COLORADO*

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The past year has seen a sharp increase in the trade in pet birds in Colorado. Accompanying this there has been a not unexpected rise in the number of cases of psittacosis in man. In contrast to the preceding 10-year period, when only one case of psittacosis was reported to the Colorado State Department of Public Health, 19 cases were reported during the first six months of 1954. The increasing number of cases reported to the U. S. Public Health Service make it clear that psittacosis is not a problem peculiar to a single state or region but one which is of national scope. The number of reported cases for the nation has risen from a relatively insignificant total between 6 and 35 each year from 1944 to 1951, to 135 in 1952, 169 in 1953, and finally to 303 during the first six months of 1954 (Table 1). The recent cases have occurred in many states (Table 2). When allowance is made for the fact that reporting of psittacosis is anything but complete, this increase assumes somewhat alarming proportions.

TABLE 1.—REPORTED CASES OF PSITTACOSIS BY YEARS IN THE U. S. A.*

Year	Number Cases Reported	Year	Number Cases Reported
1944	6	1950	26
1945	27	1951	25
1946	26	1952	135
1947	27	1953	169
1948	32	1954 (6 mos.)	303
1949	35		

*From the National Office of Vital Statistics, Public Health Service, Department of Health, Education, and Welfare.

The experience in Colorado has probably been repeated in many parts

*The serologic tests were carried out with the support of U. S. Public Health Service Research Grant, G–3802.

of the country. It represents an outbreak due in large measure to parakeets handled in pet shops and department stores and sold to individuals who had little or no knowledge that the birds might transmit psittacosis. Through the cooperation of many physicians in the state, data have been obtained on the clinical features of the illness and on the effectiveness of newer drugs, in particular, of tetracycline hydrochloride.* The purpose of this report, which is based on experience with 18 patients who acquired the disease in Colorado during the first half of 1954, is to illustrate the prevalence of psittacosis and to emphasize the fact that when psittacosis is diagnosed early and treated correctly it is now a disease of relatively slight severity.

TABLE 2.—REPORTED CASES OF PSITTACOSIS BY STATES DURING FIRST SIX MONTHS OF 1954*

State	Number Cases Reported	State	Number Cases Reported
Texas	147**	New Jersey	9
California	19	Ohio	9
Colorado	19	Connecticut	8
Pennsylvania	14	Illinois	7
New York	13	Washington	7
Iowa	9	Others	42

* From Communicable Disease Weekly Summary of National Office of Vital Statistics, Public Health Service, Department of Health, Education, and Welfare.

** For the 3-week period ending June 5, Texas reported 143 cases. The great majority of these represent cases from an epidemic among turkey processors at Corsicana.

SOURCE OF INFECTION. Fifteen of the 18 patients had been exposed to parakeets. Five worked in pet shops and had handled large numbers of birds; most of the birds appeared healthy, but all 5 patients admitted contact with some birds which had appeared ill or died. Ten patients had in their homes parakeets which they had either purchased or received as gifts. In most instances the birds were recent acquisitions. Six of the 10 stated that the birds at no time appeared ill. Four of the 10 stated that their birds either became ill and were sacrificed or had died. One patient, in addition to a parakeet contact, had pigeons, turkeys and chickens on his ranch and had recently cleaned his chicken house. The 16th patient had no known contact with psittacine birds but worked as a laboratory helper caring for pigeons which were subsequently shown to be infected. The 17th patient admitted to contact with wild pheasants. The 18th patient was not aware of any avian exposure. Data on the types of exposure are presented in Table 3.

The origin of the birds was difficult to trace because there are no regulations providing for registration or identification. Some parakeets came from breeders in the Denver area. The majority, however, appear to have originated from pet shops which obtained stock either from large distributing centers in the midwestern states or from the area adjoining the southern border of the United States.

CLINICAL FEATURES. The onset of

*Achromycin (Lederle)

symptoms was gradual in some instances, abrupt in others. Constitutional symptoms, notably severe malaise, headache, fatigability, muscle aching, anorexia, nausea and feverishness or chilliness were common presenting complaints. Shaking chills occurred in 14 of 18 patients. Most patients noted cough which usually produced little sputum. None complained of pain of pleuritic character. At least 3 became disoriented, and in one of these the degree of disorientation was such that at the time of admission to the hospital a diagnosis of encephalitis was considered.

Physical findings were in general not remarkable, and in many instances the only findings noted were the fever and prostration. Maximum temperatures ranged from 101.0° F. to 105.5° F. in the 13 patients whose febrile course was adequately recorded. In several cases careful auscultation revealed fine rales, most often posteriorly over one or both lower lobes. In 4 of the 9 patients seen by the authors a soft spleen was felt from 2 to 4 cm. below the costal margin. In one patient, during the second week of illness, scattered macular erythematous lesions appeared in small numbers on the abdomen and back.

White blood cell counts ranged from 5,500 to 11,200 per c.mm., when made during the first week of illness. Differential counts were essentially normal. Later in the course of illness total white cell counts as high as 16,000 per c.mm. were observed. Sputum and blood cultures were taken in only a few instances. No organisms were recovered which were considered to be of etiologic importance. Chest roentgenograms of 14 patients were taken; all showed areas of infiltration. These were usually ill-defined, patchy lesions involving portions of one or two lobes and were interpreted by the roentgenologist as areas of pneumonitis. The data presented above are summarized in Table 4.

TABLE 3.—SOURCE OF INFECTION

Number	*Patient*	*Sex*	*Exposure*	*Reported Contact with*			
				Sick Parakeet	*Healthy Parakeet*	*Pigeon*	*Other Birds*
1.	M.J.	F	Home	+	—		
2.	C.T.	M	Home	+	—		
3.	A.M.	M	Home	+	—		
4.	E.E.	F	Home	+	—		
5.	F.M.	F	Home	—	+		
6.	J.W.	M	Home	—	+		
7.	L.M.	M	Home	—	+		
8.	W.R.	M	Home	—	+		
9.	R.M.	M	Home	—	+		
10.	D.L.	F	Pet shop	+	+		
11.	L.W.	F	Pet shop	+	+		
12.	J.Y.	M	Pet shop	+	+		
13.	L.H.	F	Pet shop	+	+		
14.	T.A.	F	Pet shop	+	+		
15.	C.K.	M	Farmer	—	+	+	Poultry
16.	J.B.	M	Lab. helper	—	—	+	
17.	J.U.	M	Hunting	—	—		Pheasants
18.	L.F.	M	Unknown	—	—		

The duration of the febrile course ranged from 3 to 23 days and appeared to be sharply influenced by the type of therapy used. Of the 4 patients who

TABLE 4.—SUMMARY OF CLINICAL AND LABORATORY FINDINGS

Number	Patient	Age	Max. Temp. (F)	Chills	Roentgen-ray Findings	Total Wbc.	Titer of Complement-fixing Antibody* in Acute and Convalescent Sera			
							Day of Disease	Titer	Day of Disease	Titer
1.	E.E.	50	101.0°	0	..	..	3	1:8	12	1:128
2.	C.T.	51	103.0°	+	RML	10,550	5	<1:8	12	1:32
3.	F.M.	56	102.0°	0	RML	6,700	4	<1:8	9	1:16
4.	J.U.	40	104.6°	+	RLL	7,000	8	1:16	22	1:32
5.	J.B.	72	105.5°	+	LLL	7,800	14	1:8	21	1:16
6.	L.M.	38	103.8°	+	RML, RLL	12,075	12	1:8	20	1:16
7.	D.L.	37	102.2°	+	RUL, LLL	9,400	11	1:8	15	1:16
8.	L.F.	23	105.0°	+	RLL	9,500	8	<1:8	34	1:8
9.	R.M.	12	102.0°	+	RLL, LLL	7,800	3	<1:8	25	1:8
10.	J.W.	39	103.4°	+	RLL	7,150	..	..	13	1:256
11.	L.H.	46	104.0°	+	..	..	..	..	14	1:128
12.	J.Y.	40	101.0°	0	..	..	..	..	62	1:64
13.	W.R.	39	103.0°	0	LLL	4,500	..	..	46	1:64
14.	M.J.	60	105.0°	+	RLL	7,000	..	..	14	1:32
15.	A.M.	40	104.0°	+	LLL	11,200	..	..	21	1:32
16.	C.K.	51	103.0°	+	LLL	16,000	..	..	20	1:32
17.	T.A.	23	104.0°	+	..	11,300	..	..	24	1:32
18.	L.W.	40	103.2°	+	RLL	5,500	..	..	27	1:16

* Tested with Lygranum (Squibb) antigen.

TABLE 5.—COMPARISON OF RESULTS OF TREATMENT WITH DIFFERENT REGIMENS

		Penicillin			Other Drugs					
			Febrile Days				Febrile Days			
Number	Patient	Dose*	Before Started	After Started	Drug	Dose	Before Started	After Started	Total Days	Relapse
1.	C.K.	..	..	..	..	..	..	..	23	—
2.	J.Y.	..	..	..	..	..	..	..	14	+
3.	D.L.	1.4 M	9	3	..	..	..	..	12	—
4.	L.F.	2.0 M	4	3	..	..	..	..	7	—
5.	L.W.	0.6 M	18	3	..	..	..	..	21	—
6.	W.R.	0.6 M	2	11	..	..	..	..	13	+
7.	C.T.	0.5 M	3	4	OT	3.0	6	1	7	—
8.	J.W.	1.0 M	9	4	T	1.5	12	1	13	—
9.	J.B.	2.4 M	2	4	T	2.0	4	2	6	—
10.	E.E.	0.6 M	1	2	T	2.0	2	1	3	—
11.	T.A.	0.6 M	15	4	T	1.5	17	2	19	—
12.	L.H.	0.6 M	8	2	T	1.5	9	1	10	—
13.	M.J.	..	..	..	OT	?	4	3	7	—
14.	A.M.	..	..	..	OT	2.0	6	2	8	—
15.	J.U.	..	..	..	CT, OT	2.0	1	7	8	—
16.	F.M.	..	..	..	T	2.0	4	1	5	—
17.	L.M.	..	..	..	T	2.0	10	3	13	—
18.	R.M.	..	..	..	CM	1.5	4	2	6	—

* Dosages of penicillin are expressed in terms of million units per day; those of tetracycline drugs of grams per day.
OT = Oxytetracycline T = Tetracycline CT = Chlortetracycline CM = Chloramphenicol

were not treated during their acute phase or who received penicillin in amounts which were probably inadequate, the numbers of febrile days were respectively 13, 14, 21 and 23. Two of these patients had a prolonged convalescence lasting more than 2 months which was characterized by pronounced weakness and fatigability. Two had frank febrile relapses. It is interesting to note that there was no roentgenological evidence of pneumonitis at the time of relapse. No serious complications were observed, and there were no deaths in the series.

RESPONSE TO THERAPY. The data presented in Table 5 represent an attempt to compare the course of the disease in untreated patients and patients who received different drugs or combinations of drugs.

Patients 1 and 2 received either no chemotherapy or received therapy for such brief periods that they were regarded as equivalent to untreated patients. The durations of the febrile course of these individuals were, respectively, 14 and 23 days.

Patients 3 to 6 were treated with penicillin alone. Patients 3, 4 and 5 did reasonably well, and all were afebrile within 3 days after treatment was started. The amounts of aqueous penicillin administered to patients 3 and 4 were relatively large. Patients 5 and 6 were treated with procaine penicillin in smaller amounts. Patient 5 was treated on the 18th day of her illness and her improvement might equally well be attributed either to her antibiotic or to spontaneous recovery. Patient 6 (W.R.) showed very gradual improvement which was thought by his physician to have had no relationship to his therapy.

Patients 7 to 12 were treated initially with penicillin and either responded in an unsatisfactory manner or became more ill. When it became clear that they were not responding well, tetracycline or oxytetracycline was added. All patients then showed prompt symptomatic improvement with a fall of temperature to normal within 1 or 2 days. The number of febrile days after the tetracycline drug was begun was 1 in four instances and 2 in two instances.

Patients 13 to 17 were treated with only oxytetracycline, chlortetracycline or tetracycline. Four of the 5 showed prompt response with fall in temperature. The number of febrile days after treatment was 1 day in one instance, 2 days in one instance, and 3 days in two instances. Patient 15 (J.U.) showed initial improvement with fall of temperature to normal on the 3rd day after treatment was begun. Following this, however, his temperature rose again and did not return to the normal range until 4 additional days had passed. No explanation for this apparent therapeutic failure was evident. The patient differed from the others in that it was believed that his infection had been acquired from wild pheasants.

Patient 18 was treated with chloramphenicol alone in a dose of 1.5 gm. per day. He recovered promptly and became afebrile within 2 days.

CONFIRMATION OF DIAGNOSIS BY SPECIFIC LABORATORY TESTS. Complement-fixation tests with Lygranum (Squibb) antigen were performed with one or more sera from all patients. The technique used was that of the Viral and Rickettsial Disease Laboratory of the California State Department of Public Health[5]. Certain sera were tested also with antigen prepared from psittacosis virus by either the laboratories of the Hooper Foundation, University of California, through the cooperation of Dr. K. F. Meyer, or the Virus Laboratory of the Army Medical Research and Graduate School in Washington, D. C. The titers obtained at the 3 laboratories were essentially the same.

Results are presented in Table 4. Paired sera from 9 patients were tested. The antibody titers of all sera collected within a week of onset of disease were 1:8 or less than 1:8. From the 8th day on, more elevated titers began to appear. Of the 9 paired sera only 3 showed a rise of 4-fold or more in titer. The remainder showed only 2-fold increases. In several of these, the convalescent serum may have been collected before the titer had reached its highest level. The 9 patients from whom only convalescent sera were available were all found to have titers of 1:16 or more. Five of the 9 had titers of 1:64 or higher. The effect which early treatment with effective drugs may have had on the increase in antibody titer is considered in a later section.

No attempts to isolate virus from the sputum or blood of patients were made. Suspect parakeets from the homes of 3 of the patients were sent to the Virus and Rickettsia Section of the Communicable Disease Center of the U. S. Public Health Service at Montgomery, Alabama. In each instance psittacosis virus was recovered. The 10 pigeons thought to be responsible for the illness of patient J. B. were bled, and 8 were shown to have titers of complement-fixing antibody of 1:32 or more. This is accepted as adequate evidence that the flock was infected.

INAPPARENT INFECTIONS. In the course of limited testing of sera from individuals who shared the exposure of patients to birds either in the home or pet shop, 4 of 12 individuals were found to have titers of complement-fixing antibody of 1:16 or higher. None of the 4 gave a history of illness compatible with psittacosis of even mild degree and none had missed any days of work. It would appear that these 4 fall in the category of asymptomatic infections which has been previously described[7].

Discussion. In 1929 and 1930, psittacosis first assumed importance as a serious hazard to man, when epidemics originating from psittacine birds affected hundreds of individuals in Europe and the United States of America[7]. The seriousness of the disease was evidenced by the fact that approximately 20% of the victims died. Drastic restrictions were then placed on the shipment of psittacine birds on a national and state basis, and the incidence of the disease fell sharply until it came to be regarded as an exotic disease which was of relatively insignificant importance. It was well established that psittacosis virus was present in most breeding establishments, but the risk to man appeared small. That situation existed until 1951.

Meanwhile, two developments of importance had taken place. First, it had been shown that there was a huge reservoir of psittacosis or, by current usage, ornithosis virus in other avian species, notably in pigeons, doves, chickens, ducks, turkeys, finches, sparrows, canaries, various sea birds, pheasants, and others[8,16]. Many human cases were traced to nonpsittacine sources. Eradication of the virus appeared to be an insurmountable task. Second, it was shown that certain antibiotics had remarkable therapeutic effectiveness. Penicillin[4,12,15] was useful in experimental and clinical trials as was chloramphenicol[3,13]. Chlortetracycline[2,17,18] and oxytetracycline[1] appeared to be even more effective. Patients who were treated early in their course escaped with a relatively minor illness, and even those who were desperately ill could almost always be saved. The number of reported deaths from psittacosis in this country fell to a very small number[9]. When these two facts were considered along with the almost pro-

hibitive cost of enforcing adequate restrictions on the bird trade, it seemed reasonable to modify the regulations affecting interstate trade and to relax those affecting shipment from abroad. This was done in 1951 and 1952.

Since that time there has been a great increase in popularity of birds as pets. The production of birds in this country has greatly increased and, along with this, the business of smuggling birds, particularly along the southern border, has assumed massive proportions. Birds from Europe have been shipped in very large numbers to Mexico and then smuggled into this country[10]. In the latter operation they have been handled badly and have been mixed with birds of Central or South American origin which are known to be carriers of the virus; the expected epizootics of psittacosis have occurred. The volume of the trade is hard to estimate but is certainly enormous. In one Denver chain store, which had previously sold only a few hundred birds each year, the number of birds sold reached approximately 9,000 during 1953.

With the prospect of eradicating the disease so remote, the immediate problem would seem to be that of finding a way of living with it, and this can be done. Serious trouble can be avoided if the following criteria are met: (*a*) All persons acquiring birds as pets or working with them in their occupation are made aware of the fact that there is a risk that they may acquire psittacosis. While the risk may be greater with an obviously ailing bird, it is real even with a bird which appears to be in perfect health. Adding chlortetracycline to the birds' drinking water for short periods, a common practice among commercial bird handlers, will not eliminate the danger[11]. (*b*) Physicians, knowing the current increase in the incidence of psittacosis, view patients with unexplained fevers or "virus pneumonias" as possible cases of psittacosis and make inquiries regarding avian contact. The clinical features which are most helpful are the duration and height of fever, which exceed those of common respiratory infections, the greater toxicity of the patient, the occurrence of one or more shaking chills, splenomegaly when present[6], and the demonstration that the patient has one or more pneumonic lesions. The latter may be small, even though the patient is desperately ill, and it is wiser to consider psittacosis as a systemic infection rather than as a localized viral pneumonia. (*c*) Appropriate therapy is employed. While penicillin in massive doses has certainly been effective, the data presented in previous reports[12] and in this report leave little doubt that the customary doses of procaine penicillin of from 300,000 to 600,000 u/d may be ineffective. Effects with chlortetracycline, oxytetracycline and tetracycline appear to be far more predictable. This has previously been shown for chlortetracycline and oxytetracycline[1,2]. The results presented in this series, in which it was shown that each of 7 patients treated with tetracycline became afebrile in from 1 to 3 days after therapy was commenced, suggest that the latest addition to this series of drugs is as effective as the others. Intravenous therapy, in doses of 500 mg. every 12 hours, may be advisable in desperately ill patients. Otherwise 2.0 gm. per day by mouth in divided doses appears to be an adequate dose. An initial priming dose of 2 gm. may be advisable. It is possible that smaller doses will suffice.

A concluding word might be said about laboratory confirmation of the diagnosis. Definite proof can come only from isolation of virus from sputum, blood, or tissue, or from the

demonstration of a significant increase in antibody titer. Most laboratories shun the first procedure because of the risk of laboratory infection[14]. The second can be accomplished only if an acute phase serum is available, and often it is not available. One is then faced with the problem of interpreting the result of a test with only a convalescent serum specimen. When the antibody titer of this serum specimen is markedly elevated, it provides corroborative evidence in favor of the diagnosis, but is not conclusive, because antibody may have been acquired prior to the current illness. When the titer is only moderately elevated or barely above the range which may be encountered in normals, interpretation is still more difficult. This situation may occur when treatment is started early, for antibody formation may thereby be suppressed in patients with psittacosis, as has been suggested in patients with certain rickettsial diseases. The situation is further complicated by the fact that tests for psittacosis antibody use an antigen common to a large group of viruses which includes lymphogranuloma venereum in man as well as a number of viruses found in animals such as mice, cats, sheep, cattle and opossums. There will be occasional error if one interprets a single antibody determination, particularly when the titer is low, as corroborating a diagnosis of psittacosis. However, error would probably be greater if the physician should fail to make a diagnosis, even though the titer were low, when a patient with avian exposure has a clinical course characteristic of psittacosis and shows a prompt response to appropriate therapy.

Summary. 1. Clinical and epidemiologic data have been presented in 18 cases of psittacosis which occurred in Colorado during the first half of 1954.

2. Fifteen of the patients had been in contact with parakeets.

3. Treatment with penicillin appeared to be relatively ineffective unless large doses of aqueous penicillin were used.

4. Treatment with chlortetracycline, oxytetracycline or tetracycline was followed by prompt improvement in 11 of 12 patients. Tetracycline appeared to be fully as effective as chlortetracycline and oxytetracycline.

5. The problems created by the recent sharp increase in incidence of psittacosis have been discussed and the need for early recognition and appropriate treatment have been emphasized.

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THE TRANSMISSION OF INFECTIOUS MONONUCLEOSIS

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Reports from Harvard University[4], Dartmouth[15], Yale[8], the U. S. Naval Academy[2], Smith College[7] and the U. S. Military Academy[11] testify to the great incidence of infectious mononucleosis among college students; and the prevalence of mononucleosis in the Armed Forces is further attested to by many case reports which have appeared in military medical journals.

Although knowledge of the manner of transmission of a disease so common in the Armed Forces is highly desirable, actually little is known of its communicability and few hypotheses have been presented. Failures of attempts at experimental transmission and a lack of strict diagnostic criteria have added to the confusion concerning mononucleosis. The purpose of this article is to present a hypothesis of the transmission of infectious mononucleosis, based on the author's clinical observations and on a consideration of evidence presented by others.

An understanding of the transmission of this disease, and therefore why it is so common among military personnel, must begin with a critical examination of previously published material.

It is important to stress the validity of the commonly held belief that mononucleosis is most prevalent in the age group of 17 to 25 and to point out that mononucleosis is not a disease of children. Articles have appeared in the medical literature reporting mononucleosis in children, but, as will be clarified below, diagnoses were infrequently confirmed by heterophile antibody tests. My own experiences together with those of the editor of "Journal of Pediatrics" indicate that mononucleosis is rare in infants and children. "Mononucleosis" reported in children is usually an acute febrile illness clinically resembling mononucleosis in some respects: for example, in lymph node enlargement, but usually differing serologically and often hematologically. It is generally agreed that mononucleosis is rare in persons over 40 years of age.

This remarkable predilection for a narrow age span should have provided a clue as to its transmission because almost without exception acute contagious (nonvenereal) illnesses which are transmitted directly from person to person have their highest incidence in children, and there is a gradually decreasing incidence of such diseases with increasing age. Indeed, we speak of the usual contagious childhood diseases. Diseases exclusively or chiefly transmitted by food and water and insect borne diseases are, of course, not more common in children. However, the epidemiology of mononucleosis discloses no reason to suspect food or water or insects as transmitting agencies.

EXPERIMENTAL TRANSMISSION. Attempts to transmit mononucleosis to animals and humans have almost always been unsuccessful. *Listerella monocytogenes* as the cause of mononucleosis was proposed by Pons and Julianelle[18] in 1939, but was soon discarded by Julianelle[13a]; others[12] have considered an etiologic relationship be-

tween Listerella and mononucleosis highly improbable.

Julianelle and co-workers[13b] attempted to transmit mononucleosis to animals and man. Blood drawn from 14 patients between the second and seventh day of illness and from one patient on the tenth day was injected into muscles, testes, and peritoneal cavities of monkeys, rabbits, guinea pigs and mice. All results were negative. Patients gargled with 25 to 50 cc. of saline, which was collected and injected, filtered and also unfiltered, into veins, testes and peritoneal cavities of rabbits. Unfiltered washings were instilled intranasally. Results were negative. Gargle washings were filtered and then gargled for 5 minutes by 2 healthy volunteers without known history of mononucleosis, but no significant changes in health, serum or lymphocytes followed. Finally, lymph node tissue from patients with mononucleosis was ground aseptically. Filtered and unfiltered, it was injected into rabbits, but results were negative. A node was excised from a patient on the 12th day of illness and ground up; a saline extract of the node was filtered and injected into a volunteer intramuscularly without effect. A node was excised from another patient in the third week of illness; a filtered extract of the node was injected intramuscularly and also sprayed into the nose of a volunteer, but without significant result.

Attempts to transmit the disease to animals have failed, apparently because of species specificity, or because methods were faulty, or the materials used were obtained at the wrong time, or in the wrong way.

Evans[6] obtained gargle washings, sera and feces from 11 patients during the first week of mononucleosis. In his experiments, frozen and fresh unfrozen throat washings were sprayed into nose and throat with a hand atomizer, dropped directly into the nose with a pipette, and also gargled and then swallowed. It is noteworthy that throat washings were obtained by gargling with normal saline and were then mixed with equal parts of nutrient broth containing rabbit serum. Evans also introduced fecal preparations into the stomach through a tube (after excluding the presence of poliomyelitis virus and pathogenic bacteria). Serum from 9 patients was pooled and introduced intranasally, orally, hypodermically and endermally. Evans stated that strict criteria of successful transmission would include lymph node enlargement, characteristic blood changes, and a rise in heterophile agglutination titer; and he stated that he and other investigators failed to meet these diagnostic requirements.

Sohier *et al.*[20] drew blood from a patient on the tenth day of illness and injected it into a monkey. The latter showed no clinical hematologic or serologic signs of mononucleosis. Sixteen days after intramuscular and subcutaneous inoculation, blood was taken from the monkey and injected intramuscularly into a man. The latter developed the blood picture of mononucleosis, and the heterophile titer rose from zero to 1:224, 16 days after injection.

Wising[27] transferred into a volunteer 250 cc. of blood from a patient ill 13 days with mononucleosis. Twelve days after transfusion, pathologic lymphocytes were present and on the 16th day fever, sore throat and malaise were experienced. On the 24th day typical clinical and hematologic manifestations of mononucleosis were present and the heterophile titer negative before the transfusion, had risen to 1:32, at which level it stayed for over 2 months. (Neither Wising nor Sohier *et al.* used guinea pig kidney absorption tests).

De Vos and Kuipers[5] believed that they involuntarily transmitted mononucleosis by transfusing 400 cc. of blood into a patient. The latter experienced fever 21 days later, which continued for 2 weeks and was accompanied by splenic enlargement. Thirty-three days after transfusion, lymphocytes constituted 51% of the leukocytes. This figure rose to a peak of 88% forty-two days after transfusion. Many atypical lymphocytes were present; and the heterophile titer was 1:128 thirty-four days after transfusion. One week after giving blood, the donor experienced malaise and sore throat. However, when the first blood count was made, 33 days after transfusion, there were only 45% lymphocytes, albeit atypical lymphocytes were present. The donor's heterophile titer rose from 1:8 thirty-three days after transfusion to 1:64 five days later and to 1:128 after another 13 days. No guinea pig kidney absorption tests were done.

The attempts at experimental transmission to human volunteers cited above have in Evans' opinion[6] not been unequivocally successful. He stated that although the work of Wising and that of Sohier *et al.* yielded suggestive results, neither of these two fully satisfied strict diagnostic criteria: in one there was a typical clinical and hematologic course as well as a compatible lesion in a biopsied lymph node, but a negligible rise in heterophile antibody titer; in the other, a rise in heterophile antibody titer was not accompanied by any clinical, and only transient hematologic, changes. Guinea-pig kidney absorption tests might have given important confirmation. Evans stated that other workers, and in some instances even the same investigators, using similar inocula have been unable to repeat these results. Failures in experimental transmission of mononucleosis are consistent with my observations and other's which suggest that the contagiousness of even the naturally occurring disease may be low. For instance, infectious mononucleosis did not appear to spread rapidly in the close confines of a college dormitory as shown by the lack of occurrence of either clinical or subclinical illness[6]. Cross infections on open hospital wards have been notably absent. Furthermore, infectious mononucleosis is practically never transmitted to roommates, even though several days may go by before the patient is hospitalized. In the light of these observations, it is perhaps not surprising that experimental transmission of mononucleosis has not yet been established unequivocally.

The hypothesis presented below may explain the difficulties encountered in successfully transmitting mononucleosis. First, gargle washings may have been obtained too late, (although Evans secured specimens as early as the second day) or indeed too early. Patients have in my experience not knowingly had a contact with a sick person; therefore, the disease may not be transmitted by the sick, but rather by carriers. Secondly, it is conceivable, and consistent with my hypothesis, that the virus of mononucleosis is unfavorably affected by solution in normal saline, or exposure to air (abetted by vigorous gargling) or room, or lower temperatures. Therefore, the use of undiluted saliva as rapidly as possible is suggested, with little change from the normal physical surroundings of saliva. In my opinion, direct mouth to mouth contact is the way to successfully transmit mononucleosis.

De Vos and Kuipers[5] probably succeeded in accidentally transmitting mononucleosis by blood transfusion. Certainly the recipient met all criteria for a diagnosis of mononucleosis (except that no guinea-pig kidney absorption test was performed). However,

the donor's blood cell changes were not typical.

At this point a short discussion of diagnostic criteria is in order. As stated previously[11], in my opinion the diagnosis of mononucleosis should not be made without a positive heterophile reaction. A heterophile agglutination reaction may be regarded as diagnostic if a titer after guinea pig kidney absorption is at least 1:28. Leibowitz[14] stated that absorption tests were indicated whenever the antibody titer was 1:896 or lower. Bender[1], in reporting on 340 patients with mononucleosis, stated that in 5 years' experience with mononucleosis he had never seen a sero-negative case and added that it was wise to insist on strict serologic and hematologic findings. This coincides with my proposals[11] that reports of cases of mononucleosis be confined to patients meeting serologic, hemologic and clinical requirements for diagnosis.

In summarizing this section, it can be stated, that although mononucleosis may have been transmitted from man to man by injections of blood, this method of transmission is without epidemiologic importance, because patients do not acquire the disease in this manner. Experiments involving the use of gargle washings, ground lymph nodes and feces have failed. I believe that either gargle washings were obtained at the wrong time in the course of the illness, or the saliva was treated in a way to harm the agent causing mononucleosis, or both explanations may be valid.

CLINICAL OBSERVATIONS. There is an incompatibility between reports of epidemics of mononucleosis on the one hand and, on the other hand, unsuccessful attempts to transmit the disease to man and animals. Furthermore, it is hard to conceive of epidemics of a disease transmitted by droplet infection when my experiences over 6 years at the U. S. Military Academy and the experiences of investigators at many American universities agree on the virtual absence of spread among roommates or other close contacts.

There is also an incompatibility between reports of prevalence among children and my experiences in the past 8 years when, despite co-operation of pediatricians in hospitals with active pediatric services, I have rarely seen a case of mononucleosis in a child. If my hypothesis as to the transmission of mononucleosis is correct, mononucleosis should infrequently occur in children, and never in a truly epidemic form. My theory of transmission, developed below, is that mononucleosis is almost always transmitted by intimate oral contact.

Epidemics of supposed mononucleosis were more frequently written about before the Paul-Bunnell heterophile test was reported in 1932. As Evans[7] stated, the frequency of epidemics of infectious mononucleosis in a community or in institutions is difficult to determine because this disease is not usually reported to public health agencies. Yet, if one takes the published literature as a sounding board for the behavior of this condition during the last 25 years in the United States, one is struck by the fact that despite an increasingly voluminous number of reports devoted to its clinical and laboratory aspects, the reports dealing with actual epidemics of mononucleosis are singularly few. For example, in the first three decades of this century many accounts appeared of epidemics occurring in mental institutions, boarding schools, pediatric services and military camps of World War I. Yet in more recent years such reports have been few indeed.

Reports of epidemics prior to the de-

velopment of the heterophile antibody test by Paul and Bunnell in 1932 must be excluded from epidemiology inasmuch as the diagnosis of mononucleosis cannot be relied on without serologic confirmation. Typical of reports of such epidemics is that of Tidy and Daniel[23] in 1923, who reported an epidemic (in a school) involving 25 boys between the ages of 8 and 13 years. These boys had lymph node enlargement, but hematology itself cast doubt on the diagnosis, in that only one patient's white blood cell count contained over 60% lymphocytes and only 6 additional patients had over 50% lymphocytes. Furthermore, typical of reports of epidemics, clinical manifestations were not those usually observed; and constitutional symptoms and pharyngitis were not prominent.

An epidemic of what was believed to be mononucleosis was reported involving 16% of 108 children in a sanatorium[19]. Clinical manifestations were absent. Blood counts uniformly revealed leukocytosis and lymphocytosis. White blood counts ranged from 18,000 to 59,000. In all 16 cases the heterophile test was negative.

Another epidemic was reported by Nolan[16] in 1935. He reported on 222 patients; almost all were children. In only 40 instances were heterophile tests done. Readings were made after overnight refrigeration, a technique shown by Stuart *et al.*[22] and Zarafonetis and co-workers[28] to yield many falsely positive results. The guinea-pig kidney absorption technique was not used. The symptom complex reported was most unusual for mononucleosis: fretfulness, change in personality, fever, pain in neck. There was no mention of physical signs; and it appears that many patients were never seen and none were examined by the author while they were sick. Hematology disclosed a startling increase of abnormal lymphocytes; and "lymphoblasts" were seen in 35% of cases, with "abnormal monocytes" reported in 80%. Details of cases were not given. It is highly questionable whether this was an epidemic of mononucleosis. The description is inadequate to substantiate a diagnosis of this disease; and the blood cells reported are not common in mononucleosis. This report shows once again that series with a high incidence of unusual symptoms or mild or subclinical cases contain a low incidence of positive heterophile antibody reactions.

Goldthwaite and Elliot[9] reported what they termed a mild clinical and subclinical epidemic "most probably of infectious mononucleosis" in a small detachment of men. During the course of a winter every member of a group of 26 men showed atypical lymphocytes in his blood smear. However, symptomatology was not characteristic of mononucleosis in that, except for the original patient, only 1 of 4 clinically sick patients had sore throats. There were 21 subclinical cases. Again it is noteworthy that only the original case had a positive heterophile antibody reaction, and guinea pig absorption was not reported in this case. This "epidemic," like those previously cited, cannot be considered as meeting diagnostic requirements for mononucleosis.

Vander Meer, Lutterloh and Pilot[24] reported 26 clinical and 340 subclinical cases in an Army camp occurring between December, 1943, and May, 1944. Of these 366 cases, there were only 31 instances of positive heterophile agglutinations; once again, guinea-pig absorption tests were not done. Only 18 of these patients had characteristic blood findings and clinical manifestations. Of the 340 subclinical cases, only 13 had positive heterophile antibody reactions. These investigators also examined blood smears of 600 men in a

military unit because several of their clinical cases came from this unit: 41% of smears of 522 men tested had occasional atypical lymphocytes. Only 74 heterophile tests could be done on this group; 13 were termed positive reactions, that is, positive in dilutions of 1:56 up to 1:224; however, no absorption tests were done.

In only two epidemics did a relatively large percentage of patients have positive heterophile antibody reactions. However, in both groups heterophile tests after guinea-pig absorption were not performed. In the first report[10], 1000 heterophile antibody tests were done in 3 weeks and results were read after overnight refrigeration, a technique which yields falsely positive results. In the second report[26], the technique of the heterophile test was not stated. The first of these epidemics, occurring in a hospital in Britain in 1942, was reported by Halcrow, Owen and Rodger[10], who diagnosed mononucleosis by performing blood counts on 296 individuals, all patients in, or staff members of, a hospital. One patient was an 84-year-old woman, another was 64. Not only did 97.9% of the 296 show abnormalities which, it was thought, indicated mononucleosis, that is, either hematologic or clinical manifestations, but very many relatives of staff members had abnormal blood counts. Clinical manifestations were termed protean; and the disease process was unaccompanied by symptoms in 165 cases. Clinical manifestations were unusual in that fever was not a feature except in 4 "anginose" cases, and sore throat occurred in only 35 instances. Hematology was unusual in that well over half of the patients had less than 50% lymphocytes in their blood smear and in only a few cases did lymphocytes exceed 75%. This combination of predominantly subclinical cases, unusual symptoms in clinical cases, relatively low lymphocytosis (that is, compared with blood findings described in most reported series) indicate that this may not have been mononucleosis, an indication supported by the use of a faulty heterophile antibody technique and the absence of guinea-pig absorption tests. Inasmuch as these patients were members of a war-time hospital population, it is possible that the heterophile agglutinations, and perhaps blood cell changes also, may have been responses to inoculated materials: horse serum or others. It was not stated which inoculations these patients had received. At any rate, except for the next report, spread of mononucleosis through the population of a hospital has never been duplicated in any American hospital, despite the fact that there are many more hospitals in this country and that patients are usually, if not always, allowed to remain on open wards.

The epidemic reported by Wechsler, Rosenblum and Sills[26], which occurred on an Army post between August, 1943, and February 29, 1944, is similar to the epidemic described by Halcrow *et al.*: 20% of the cases were not ill. Many unusual symptoms were encountered. Cough was common and pulmonary signs were present in 30% of the cases. A case was not included unless "leukocytoid" lymphocytes constituted at least 10% of the total white blood cell count, except that cases not meeting this requirement were included if the heterophile reaction was positive in a dilution of 1:224 or above. No guinea-pig absorption tests were performed. The technique of the heterophile test was not stated. Strikingly similar to the British epidemic, it was found that during the height of the epidemic practically every patient in the hospital showed varying percentages of abnormal lymphocytes in blood smears; and the authors believed that many soldiers

on the post had mononucleosis but were without clinical manifestations and hence not hospitalized. White blood counts of 68% of patients showed only 49% or less lymphocytes. Once again the combination of large numbers of subclinical cases, unusual symptomatology and relatively low lymphocytosis is seen. This report differs from reports of other epidemics in that 83% of the patients had heterophile titers of 1:56 or higher. However, guinea-pig absorption tests were not performed, except at random (number not stated). In some cases the tests were confirmatory, in others they were not. The authors suggest that the heterophile antibodies in an epidemic differ from those described for sporadic cases. This is another way of saying that possibly the antigen (that is, the causal agent) was not the same in this epidemic as in sporadic cases. This conjecture supports my previously expressed belief that we have perhaps considered closely allied diseases caused by closely related micro-organisms as one disease, mononucleosis.

Stevenson and Brown[21] reported a series of 123 patients with supposed mononucleosis. Most of these had no clinical manifestations of disease. Sera from all persons investigated were examined for heterophile antibodies using absorption techniques. Only 16% had serologic proof of mononucleosis. Interestingly, although the great majority of patients clinically ill with apparent mononucleosis had positive heterophile titers, only 7% of the subclinical cases had such titers. Only 2 of the 123 patients were children; it is not plain whether or not they were in the serologically proven group.

Watson and co-workers[25] reported that of a group of 102 medical students supposedly in good health, 55% had atypical lymphocytes during a three-week period of study. However, only 4% had positive absorbed heterophile titers of 1:28 or more at the initial bleeding. Inasmuch as none of these students were sick, it was surprising to find that after three weeks 27% had positive blood titers. Only 9% had absorbed titers of 1:56 or higher. The technique of the heterophile test was not given.

Since the apparent lack of contagiousness of mononucleosis might be explained by subclinical infections, I questioned and examined 10 cadets who were roommates of patients with mononucleosis. All of these contacts had heterophile tests within one month of the roommates' admission to the hospital and three also had hematologic studies. Heterophile antibody tests were negative in all instances; one contact's white blood cell count showed 63% lymphocytes, of which 1% were atypical.

Inasmuch as all reports of so-called epidemics have mentioned large numbers of subclinical cases, it occurred to Evans[6] that possibly for every clinically recognized case encountered in universities there might be many others with inapparent infections, and that the sporadic nature of infectious mononucleosis might be more apparent than real, since these very mild cases might be undetected. Therefore he looked for inapparent infections in a dormitory in which 5 clinical cases had occurred within one month. The environmental circumstances were such that ready transmission of a highly contagious disease to susceptible persons would be expected. On this basis and with the knowledge that cervical adenopathy, pathologic lymphocytes and high heterophile antibody titers usually persist for several weeks after onset of symptoms, a survey of all three units, including house supervisors and kitchen personnel, was carried out. The 5 patients and 58 other individuals lived

in a dormitory consisting of three housing units (each housing unit had separate kitchen and dining facilities). Each patient was interrogated for symptoms; cervical lymph nodes were palpated; hematologic studies and tests for heterophile antibodies were done. Lymphocytes and monocytes constituted over 50% of the total leukocytes in only 3 of the 58 dormitory residents. In these 3 cases cervical lymph node enlargement was absent and the heterophile test was negative. Atypical lymphocytes were present in only one blood smear. Two residents had faintly, but not significantly, positive heterophile tests but no hematologic or clinical evidence of mononucleosis.

Finally, another convincing example of the noncontagiousness of mononucleosis was given by Oglesby Paul[17] who, on a destroyer, discovered 3 cases of mononucleosis within one week. All 3 patients had typical clinical and hematologic manifestations of mononucleosis, and 2 had diagnostic heterophile test results. In the third case a negative heterophile titer was found on the fourth day of illness, but the diagnosis was confirmed later at a naval dispensary. Paul stated that the living conditions on board a destroyer are favorable to the progress of an epidemic: "The members of the crew live in necessarily crowded quarters, with constant close contact with their bunk neighbors. In the chow line, mess hall, crew's head, gun mounts, engineering spaces, and elsewhere, they live and work shoulder to shoulder. The common cold and its variations characteristically run through the entire crew, once introduced on board."[17]

Despite the existence of such conditions favoring the spread of any infectious disease, only the 3 cases of mononucleosis occurred, all diagnosed within one week. Two were treated on board in their own bunks throughout the entire course of their illness; the third was on board for the first 5 days. A careful check during the ensuing 3 months, as well as a review of the complaints of all men who had reported to the sickbay in the previous month, failed to show indications of involvement of any other member of the crew. The ship had visited a liberty port approximately 5 weeks before, but thereafter operated in an area which precluded granting any liberty. It is rather remarkable that the 3 cases reported should have involved men with entirely different duties and with entirely different living quarters offering the least opportunity for physical contact. To check on the possibility of subclinical cases, Paul obtained hematologic and serologic examinations of 5 additional men; results were negative.

PERSONAL OBSERVATIONS. In over 6 years at the U. S. Military Academy, I saw all patients with mononucleosis. The endemic nature of mononucleosis soon became apparent. It was puzzling that whereas roommates readily acquired respiratory diseases from each other, I never saw mononucleosis in roommates. This experience was, of course, in accord with the views of Evans, Paul and others expressed above.

Transmission by food, water and insects was easily excluded. Next I investigated the possibility of transmission by sexual relations; however, it was definitely concluded that several patients had never had sexual relations.

I noticed that more cases were encountered in February and August than in other months. Despite interrogations of many patients, I could never establish a source of infection until a patient became ill on February 8, 1951. He stated that on December 23, 1950, he was on a train and spent about twelve hours in the company of a female medical student whom he had

never seen before and whom he never saw again, but with whom he corresponded. These 2 individuals kissed frequently and, more important, in a way to allow mingling of saliva. They also carelessly drank from the mouth of a bottle passed around in a circle of train acquaintances. The patient learned, by letter, from his train acquaintance that she was a patient in a university hospital with a disease diagnosed as infectious mononucleosis, and that the onset of her symptoms antedated the onset of his symptoms by two or three days. It seems likely that either the patient and his train acquaintance were infected by drinking from the same bottle, or that the woman may have acquired the mononucleosis virus in an oral contact with someone else on the train and passed it on to my patient.

If in fact my patient had acquired mononucleosis 47 days before his first symptoms began, this long incubation period was at variance with surmises as to incubation periods of one to two weeks expressed in texts. Further inquiries were then made to support the concepts of long incubation period and transmission by intimate oral contact.

Since February, 1951, I have obtained a history of oral contact, 32 to 49 days before onset of symptoms, from all of 73 patients with mononucleosis, except two. One exception was a chaplain's assistant who stated he frequently drank soft drinks from bottles passed to him by friends. Obviously, just as many young persons without mononucleosis might give similar contact histories. Yet, there is further circumstantial support for this hypothesis. Three patients stated that they had engaged in intimate kissing only once: contact had taken place between 42 and 45 days before onset of symptoms. The relatively high incidence in February and August is explainable by this theory, since about 45 days previously, cadets were having semi-annual vacations, and therefore were having maximum opportunities for contact. They had relatively few opportunities to acquire mononucleosis during the academic year because, unlike civilian collegians, they were usually confined to their rooms in the evenings. Interestingly Evans[7] stated without comment, that highest monthly peaks occurred in the months following the end of the summer, Christmas or spring vacations.

The concept of an incubation period of about 40 days is strengthened by circumstances mentioned by Paul[17]: his three patients developed mononucleosis about the same time, although they had been on board a ship for 5 weeks since their last visit ashore. Another case involving a sailor is pertinent. This man developed mononucleosis after he had been on board ship for two months except for shore leave on the 42nd, and later on the 37th day, before the onset of illness. During both leaves he had had intimate oral contacts. It is likely that naval medical officers, aware of circumstantial evidence discussed in this paper, shall be able to confirm the concept of a relatively long incubation period.

The hypothesis of intimate oral transmission serves to explain many observations. It explains why the disease is not transmitted to roommates. It explains why attempts at experimental transmission have been unsuccessful: presumably the agent of mononucleosis does not survive outside of the human body.

The hypothesis explains why serologically proven cases of mononucleosis in children are infrequently, or rarely, seen.

The hypothesis also explains why I have rarely seen mononucleosis in a

married person: unmarried persons are much more likely to kiss promiscuously. It explains why precisely the age group most active in osculation, about 17 to 26, provides the bulk of mononucleosis patients. Although I have seen well over 200 cases of mononucleosis since 1946, I have rarely seen the disease in a person over 30 or in a child under 14 (despite the cooperation of alert pediatricians).

The proposed hypothesis of transmission explains the epidemiologic discrepancy observed in hospitals: young nurses, medical students and interns acquire mononucleosis relatively often, yet cross infections on open hospital wards do not occur. The reason, of course, is that the disease is not acquired from the patients on the wards.

It is, however, uncertain whether mononucleosis is acquired from patients or from carriers. One of my patients was a young woman who was engaged to a student. She had had oral contact only with this one young man. The woman had clinically, hematologically and serologically typical mononucleosis; however, the male contact's lymphocytes and serum were found to be normal. The contact may have been a carrier. Unfortunately, although questioned about symptoms of illness during the preceding months, he was not questioned about illnesses in the less recent past. Recently a young woman patient was admitted with mononucleosis. She had had oral contact with only one man in the preceding 4 months. His blood cells and serum were tested and found to be normal. Yet questioning revealed that, one year before, he had had an obscure febrile illness called a "virus" illness, which lasted one week. Recently a soldier was admitted with typical mononucleosis. He stated that he had kissed only one woman in the past half year; his oral contacts with her were frequent and intimate. Questioning the woman revealed that she had had an illness 13 months before the soldier's acquiring mononucleosis characterized by fever for 8 days, moderate headache, chilliness and sore throat. Her physician diagnosed this illness as "grippe." Examination of her blood cells and serum, after the soldier became ill, revealed normal results. Possibly both of the above mentioned contacts of patients had had mononucleosis which was misdiagnosed; and possibly, just as hepatitis patients may harbor the virus of hepatitis months or years, so mononucleosis patients may remain carriers for indefinitely long periods. The excretion of mononucleosis virus may be intermittent. Possibly either chronic convalescent carriers or passive carriers, who have never had clinically apparent mononucleosis, are involved in the transmission of the disease. At any rate, my observations have led me to believe that infectious mononucleosis is usually transmitted as a result of intimate oral contact which permits a transfer of saliva. Indirect transfer of saliva (mediated by an object which is quickly passed from mouth to mouth) is probably the mode of transmission in a minority of cases, especially when children are affected.

Summary. 1. The experimental transmission of mononucleosis has been almost uniformly unsuccessful. Possibly the disease may be experimentally transmitted by injecting blood of patients who are in the incubation period.

2. Mononucleosis rarely occurs in roommates or as a cross infection on open hospital wards.

3. Reports of epidemics before the discovery of the heterophile antibody reaction are undependable because it is uncertain whether the diseases described were in fact mononucleosis. Epidemics reported since 1932 are

characterized by an abundance of subclinical cases and usually by a paucity of positive heterophile reactions. It is proposed that these epidemics have been instances of a disease, or diseases, related to, but not identical with, infectious mononucleosis.

4. Sporadic instances of unquestionable mononucleosis in children are rare; and reports of epidemics among children are unreliable.

5. The hypothesis is proposed that mononucleosis is transmitted chiefly by direct intimate oral contact which allows for salivary exchange, and that the incubation period of the disease is about 33 to 49 days.

6. This hypothesis explains the unusual age incidence, the increased seasonal incidence (that is, after vacations) in collegians, the fruitless results of attempts at experimental transmission, and the absence of transmission to room and ward mates.

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VARIATIONS IN HISTOCHEMICAL FINDINGS IN LIVER TISSUE OBTAINED BY BIOPSY

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During previous investigations[2], appreciable variations in the distribution of histochemically demonstrable lipids, parenchymal fatty cysts, and glycogen-vacuolated nuclei were sometimes noted in liver biopsies. Rather than being intralobular, these variations occurred between different, larger regions of one or more sections cut from a specimen of liver obtained with a Franseen needle.

Striking changes in the distribution of fatty cysts and histochemically demonstrable lipids in the liver biopsy specimen were noted in a 30-year-old, obese, female diabetic patient who had been informed 3 years previously that her blood sugar was high, but who had not taken insulin until 13 days prior to biopsy. Admission to hospital was because of migratory pain, swelling and tenderness in the left knee, wrist and right ankle, accompanied by fever and malaise of 2-weeks' duration. At the time of admission only slight swelling and tenderness of the right ankle persisted. The liver was not enlarged. She also had latent syphilis. Further details regarding the clinical and laboratory data on this patient may be found in a previous communication[2], (Patient No. 10). Despite thorough investigation, the cause of the arthritis was not determined. Histologic examination of the liver biopsy specimen revealed parenchymal cell degeneration, focal lesions probably of necrotic origin, portal fibrosis and mononuclear cell infiltration of portal areas. Granulomatous lesions compatible with sarcoidosis were also present. The cytoplasmic glycogen was graded as *2* (range *0* to *4*), and showed marked variation in distribution in the specimen. The incidence of nuclear glycogen-vacuolation was 3%. Demonstrable cytoplasmic lipids were graded as *2* and occurred as small and medium-sized, neutral droplets and occasional acidic ones. Large parenchymal fatty cysts were also graded 2 and contained neutral lipids and fatty acid crystals. The striking feature was the variation in distribution of lipids (Fig. 1). Many liver lobules were scarcely affected by the fatty change

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whereas in another portion of the biopsy specimen the changes were striking. It is possible that, if the needle had missed either of these two regions, erroneous conclusions concerning the presence or absence of fatty metamorphosis in the liver would have resulted.

In some of our patients, nuclear glycogen-vacuolation also presented slight regional variations which had to be considered in the over-all assessment of this change. Striking nonuniformity was noted in a 64-year-old woman (Patient No. 1, previous communication[2]), who had had symptoms suggesting diabetes mellitus for one year. The liver edge was 5 cm. below the costal margin in the right midclavicular line and not tender. She had never received insulin prior to the liver biopsy. The specimen showed focal lesions of necrotic origin and some parenchymal cell degeneration. Cytoplasmic neutral fat droplets, generalized in distribution were graded as *4*. Parenchymal cysts containing neutral fats were graded as *2*, some of which contained fatty acid crystals. The cytoplasmic glycogen showed marked variation being graded 3½ in one portion of the biopsy and ½ elsewhere. The incidence of nuclei showing glycogen-vacuolation ranged from 2 to 30% in different parts of the same specimen, varying reciprocally with the cytoplasmic glycogen grading.

In order to obtain further information concerning variations in histochemical findings in liver tissue, two separate specimens were removed from three livers during the course of operation. The biochemical, histologic and histochemical methods employed in this study are identical with those previously described[2].

Case Reports. CASE NO. 1. A white man, 37 years of age, with chronic duodenal ulcer disease was admitted to the Graduate Hospital, January 24, 1953, because of hematemesis. He was not an alcoholic and not obese. Shock was not present prior to operation. The liver was not palpable. Preoperative hematological findings, diet and blood transfusions were as follows:

Date January 1953	*Red Cell Count (mill. per c.mm.)*	*Hemoglobin (gm. per 100 cc.)*	*Diet Prescribed*	*Blood Transfusions (cc.)*
24	..	13.2	4½ oz. whole milk hourly x 12 12 oz. prune juice daily	500 ..
25	..	11.7	As above	..
26	3.95	11.8	As above	..
27	..	9.0 to 12.3	As above	1500

On January 28, a subtotal gastrectomy was performed. During the operation 2 needle biopsies were taken from the exposed liver. The schedule was as follows:

A.M.	
10:05	Operation commenced. Anesthesia: pentothal, cyclopropane, ether and oxygen
10:10	Intravenous 5 per cent glucose in water started. Total given, 300 cc.
10:25	First liver biopsy: anterior aspect of right lobe
11:00	Blood transfusions started; 1300 cc. given
P.M.	
1:25	Second liver biopsy, anterior aspect of right lobe
2:37	Operation completed

Histologic examination of both liver specimens revealed a slight increase in connective tissue in the portal areas and some focal lesions. The histochemical observations are recorded in Table 1. The intracellular lipid droplets were graded as *1½* in the first specimen. They were mostly fine and acidic, al-

though occasional ones were large and neutral. In the second specimen, taken 3½ hours later, the lipid findings were similar but graded 2, an insignificant difference. There were no fatty cysts. The cytoplasmic glycogen was uniformly distributed and graded 3 in the first specimen but was definitely decreased in the periportal regions with an over-all grade of 2½ in the second. Many factors such as anesthesia, surgical intervention, and intravenous glucose and blood, were active during the 3½-hour interval and may have influenced the decrease in the amount of glycogen in the periportal cells.

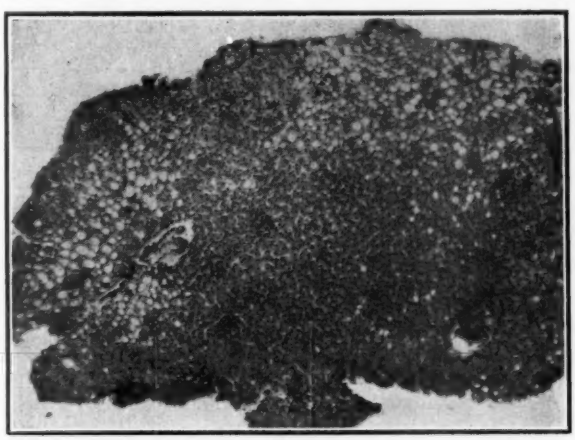

Fig. 1.—General view of microscopic section showing irregular distribution of fat vacuoles in the liver. Paraffin section (4 micra). Hematoxylin and Eosin. × 35.

CASE NO. 2. This 38-year-old woman with primary biliary cirrhosis was studied extensively by Dr. H. J. Tumen. Her serum lipid concentrations, determined in the laboratory of Dr. I. J. Pincus, were: phospholipid phosphorus, 59.7 mg.; total cholesterol, 1880 mg., and free cholesterol, 508 mg. per 100 cc. During laparotomy at the Albert Einstein Medical Center, Northern Division, Philadelphia, a few days later, her liver was found to be enlarged, green and smooth. No abnormalities were found in her extra-hepatic biliary system.

Two liver specimens were obtained: a needle biopsy from the anterior aspect of the right lobe and an excised one from an adjacent area 25 minutes later. In the first biopsy (Table 1), a few fine to large droplets were found. They were mostly neutral, occasionally acidic, and occurred in a few parenchymal and Kupffer cells and among some connective tissue fibers. In the surgical specimen, the over-all amount of lipids did not differ appreciably but, in some areas, the parenchymal cells contained quite a few very fine droplets while elsewhere, they contained none. The liver lipids may have undergone evident change during the 25-minute interval, or the needle may have sampled only a "fatty" region and missed a "non-fatty" one. The cytoplasmic glycogen was graded 1½ in the first specimen (Fig. 2). There was some intralobular variation in its distribution and none was found in the cells adjacent to the bands of fibrous tissue. In the surgical specimen (Fig. 3), there was more glycogen (grade 3) and it was more uniformly distributed, although there was still none in the cells near the connective tissue bands. Such a demonstrable change in liver glycogen could have occurred within 25 minutes

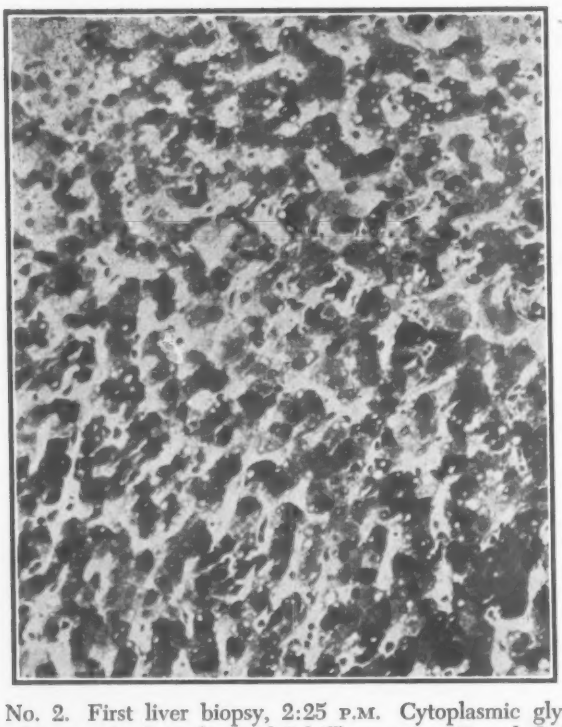

Fig. 2.—Patient No. 2. First liver biopsy, 2:25 P.M. Cytoplasmic glycogen, grade *1½*. Paraffin section (4 micra). Periodic acid-Schiff's reaction. B and G filters. × 200.

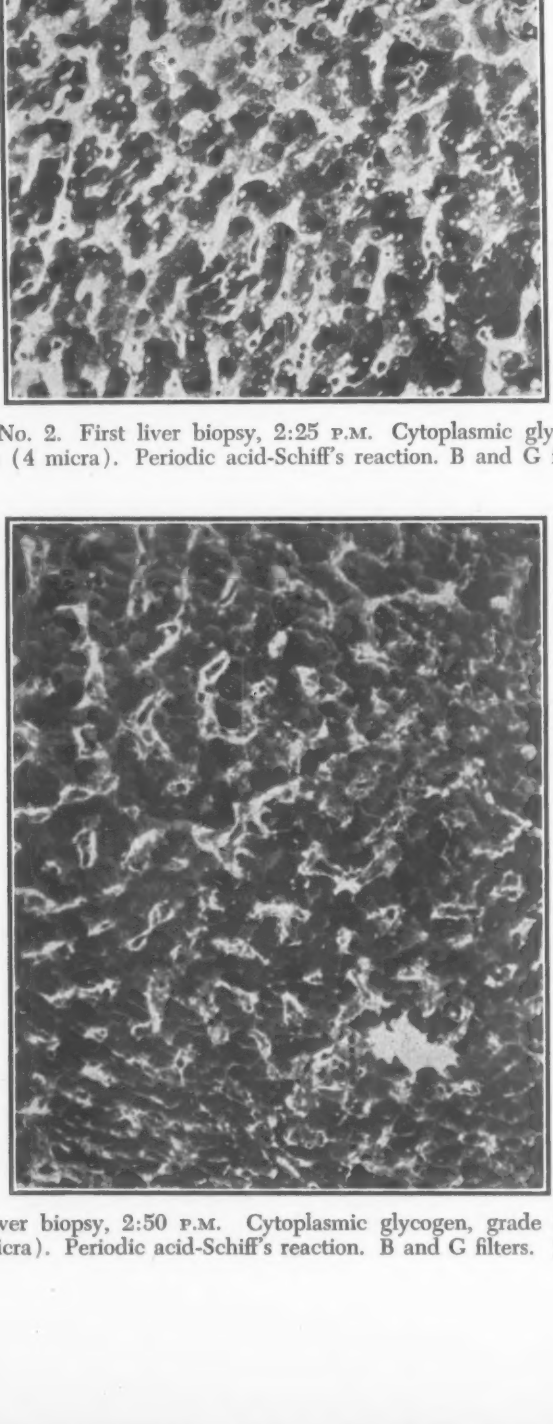

Fig. 3.—Second liver biopsy, 2:50 P.M. Cytoplasmic glycogen, grade 3. Paraffin sections (4 micra). Periodic acid-Schiff's reaction. B and G filters. × 200.

since glucose was being administered intravenously throughout the operation.

CASE NO. 3. This 72-year-old white male was admitted to the Graduate Hospital with a one-year history of persistent icterus, pruritus, anorexia and progressive loss of 37 pounds in weight. He was 5 feet tall, weighed 103 pounds and was approximately 29% underweight. The firm, round, nontender edge of his liver was 10 cm. below the costal margin in the midclavicular line. The surface of his liver was smooth. There were no other signs of hepatic disease. The direct reacting serum bilirubin was 19.8 mg. and the total 24.8 mg. per 100 cc. The total serum lipids were 2120 mg. per 100 cc., serum phospholipid phosphorus, 62.8 mg. per 100 cc., and serum fatty acids 44.2 mEq./L. Total serum cholesterol was 595 mg. per 100 cc. The latter three determinations were made in the laboratory of Dr. I. J. Pincus. The total serum proteins were 7.52 gm., albumin 3.45 gm., and globulins 4.07 gm. %. Cephalin-cholesterol flocculation, thymol turbidity and flocculation tests, serum colloidal gold and scarlet red tests were consistently normal. Serum alkaline phosphatase activity was 50.0 Bodansky units. A diagnosis of extrahepatic obstructive jaundice with secondary biliary cirrhosis was made. The diet prescribed in hospital provided approximately 170 gm. protein, 115 gm. fat and 365 gm. carbohydrate. At laparotomy a mass which was considered to be carcinoma of the ampulla of Vater was found. There were no metastases in the enlarged firm green liver.

A needle biopsy was taken from the anterior aspect of the liver and immediately afterwards a small surgical specimen was excised from an adjacent, apparently similar region. The histological findings were those of secondary biliary cirrhosis with slight portal cirrhosis. These changes were similar in both specimens. The histochemical findings are given in Table 1. There was a great difference between the histochemically demonstrable lipids in the two portions. Whereas the probably neutral, intracellular lipid droplets were graded as *f. tr.* and occurred as medium-sized droplets in scattered cells in the first specimen, those in the surgical one were graded as 2½, occurred in most cells and were usually large, acidic, and parasinusoidal.

Comment. Needle biopsy of the liver has been used for some years as an aid to diagnosis and, more recently, for certain biochemical and histochemical studies. Although the information which it provides may be inaccurate when the lesions are focal, the small cylinder of hepatic tissue so obtained has been assumed to be representative of the entire organ in diffuse diseases of the liver. Deane, Nesbitt and Hastings[3] and Gomori and Goldner[4] were among the first to demonstrate that even in such conditions, the histological and histochemical findings may vary in different regions. The findings of Waldstein and Szanto[6] tend to favor the original assumption of a uniform distribution of most histologic changes in diffuse liver diseases. However, as shown by our cases, significant variations in the distribution of parenchymal fatty cysts, cytoplasmic fat droplets or glycogen-vacuolated nuclei may be observed in needle biopsy specimens.

Although the conclusions of experimental studies may not be applicable to humans, Pincus[5] did observe definite and sometimes appreciable quantitative variations in the distribution of lipids as determined biochemically in different regions of the fatty livers of pancreatectomized dogs. Billing and her associates[1], on the other hand, found "relative uniformity in the distribution of lipids throughout the human liver" and a "good correlation between histological and chemical findings" in comparable studies on needle biopsies of human livers at necropsy, including one from a patient who died in diabetic coma. That significant histochemical variations in lipids may be found in different specimens of the same liver is well shown by our case No. 3. Although no such differences

TABLE 1.—FACTORS AFFECTING HISTOCHEMICAL FINDINGS

			*Histochemical Observations**						
			*Lipids***		*Glycogen†*		*Nucleic Acids‡*		*Iron Pigm.††*	
Case	*Histological Diagnosis*	*Time of Biopsy*	*Cytopl. Droplets*	*Cysts*	*Cytopl.*	*Nucl. Per Cent*	*Nucl. DNA*	*Cyto. PNA*	*Paren.*	*Kupf.*
1.	Portal fibrosis	10:25 A.M.	1½ fine, ac.; few lg., neut. (1)	0	3 unif.	0	1-3 mkd. varn.	2	0	0
		1:55 P.M.	2 fine, ac.; few lg., neut. (1) (2)	0	2½ decr. port.	0	1	2	*f.tr.*	0
2.	Primary biliary cirrhosis	2:25 P.M.	*tr.* fine, neut., occas. ac., scat. genl. and trabec.	0	1½ unif., but none near trabeculæ	0	3	3	0	0
		2:50 P.M.	*tr.* fine, neut., occas. ac., reg. varn. (see text)	0	3 unif., but none near trabeculæ	0	3	2	0	0
3.	Secondary biliary cirrhosis with slight multilobular cirrhosis	2:45 P.M.	*f.tr.* med., neut., scat.	0	1½ mkd. varn.	2.7	2	2	0	0
		2:50 P.M.	2 lg., neut. and ac. parasinusoidal	0	1½ mkd. varn.	2.8	2	2	0	0

* *Grading for observations on lipids, glycogen, nucleic acids and iron pigment,* ranges from 0 to 4 and includes within the interval 0 to 1, *v.f.tr.* (very faint trace), *f.tr.* (faint trace) and *tr.* (trace).

** *Lipids*: Separate data are given for cytoplasmic fat droplets and for fatty cysts as follows:
Size: med. = medium; lg. = large
Reaction: ac. = acidic; neut. = neutral; cryst. = crystals also present
Distribution within hepatic lobules: genl. = general; port. = periportal; cen. = central; scat. = scattered; trabec. = intratrabecular
(1) in most of remaining cells (2) difference not significant

† *Glycogen: Cytoplasmic*—Distribution: unif. = uniform; varn. = variation; mkd. = marked; mod. = moderate; decr. port. = decreased in peri-portal regions; sl. decr. = slight decrease. *Nuclear* = percentage of nuclei containing some glycogen.

‡ *Nucl. DNA* = Nuclear desoxypentose nucleic acid. *Cyto. PNA* = Cytoplasmic pentose nucleic acid.

†† *Iron Pigm.* = Iron pigment. *Paren.* = Parenchymal cells. *Kupf.* = Kupffer cells.

were found in Patient No. 1, slight but definite ones were noted in Patient No. 2. Thus it may be possible to draw erroneous conclusions from histochemical studies of lipids in liver tissue obtained by needle biopsy. Such variations may indicate that only portions of the liver are active at any given time, perhaps depending upon functional changes related to the patency of the many vessels carrying blood to the cells of this complex organ. As a result, some regions may be more susceptible to injurious effects than others. Structural vascular changes might be expected when the liver architecture is disrupted as in patients with well marked portal cirrhosis. In none of our patients, however, was there any striking degree of lobular disorganization.

Conclusion. Our investigations have shown that appreciable differences in histochemically demonstrable lipids and in glycogen-vacuolation of nuclei may sometimes be found in portions of the same specimen of liver tissue obtained by needle biopsy, and that similar variations may exist in different biopsy specimens of the same liver. The recognition of this possibility is important in the assessment of histochemical findings in liver tissue. These variations may signify that portions of the liver differ in their functional activity at any given time.

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IN VIVO MICROSCOPIC OBSERVATIONS OF THE CIRCULATING BLOOD IN ACUTE MYOCARDIAL INFARCTION*

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This study was undertaken to add to the objective tests[10,18,36] which evaluate anticoagulant therapy in acute myocardial infarction[13,14,26,30-33,40,41]. At the present no test determines the fluidity of the blood at the microscopic level *in vivo*. Neither are there any tests which will objectively determine therapeutic questions like the following: Does the anticoagulant revert the composition of the blood to its healthy fluidity? What is the optimal concentration of anticoagulant to use in preventing microscopic thromboembolism? And, how long should anticoagulant therapy be maintained?

To answer some of these questions, a microscopic indicator, a stereobinocular microscope, was brought to bear on a statistically valid sample of arterial blood *in vivo*. The indicator was applied to the blood which circulated through the bulbar conjunctiva. This site has been found to be most satisfactory for studying the circulation with the microscope in man[22]. No treatment is needed to make the blood vessels visible. The vessels are parallel to, and near the surface, which makes it possible to trace the flowing column of blood from the arterial through the capillary and into venous channels without interruption. The tissue is bathed by its physiologic fluid, the tears, and there are exposed and protected areas available for study. Most important, the blood flowing through the arterioles of the bulbar conjunctiva represents a statistically valid sample of *all* of the arterial blood in the body. This fact has been repeatedly demonstrated in many different species ranging from amphibians to primates[3,19-21]. No microscopically visible differences have been detected between the arterial blood in the bulbar conjunctiva from the arterial blood in internal organs (gastrointestinal tract, striated muscle, brain, and others) *in the same animal*[3,20,22].

The general plan of this study consisted in quantitatively evaluating the intravascular reactions of the circulating blood and walls of blood vessels in several kinds of patients. The controls consisted of healthy subjects and patients with cardiac lesions, angina pectoris and inactive rheumatic heart disease with mitral stenosis. The patients with myocardial infarction fell into three categories: those who had no treatment; those treated with heparin and Dicumarol (3,3′ methylenebis-[4-hydroxycoumarin]); and those treated only with Dicumarol or Tromexan (3,3′ carboxymethylenebis-[4-hydroxycoumarin] ethyl ester). Each subject, whenever possible, acted as his control

*This study was supported by a grant from the American Heart Association, and the Life Insurance Fund.

which was accomplished by observing the reactions prior to, during, and after anticoagulant therapy.

Materials and Methods. The patients used in this study were from the staff and private services from the University Hospitals of Western Reserve University*. The diagnoses were made by the physicians in charge. The patients with acute myocardial infarction from which the results were tabulated satisfied the criteria recommended by the Criteria Committee of the New York Heart Association[29].

THE TECHNIQUE OF STUDYING THE CIRCULATION. The patients were studied in the dorsal recumbent position. The eye was held "still" by having the patient "fix" his gaze on a small spot which had been placed in such a position that the upper eyelid had to be elevated, thus exposing the maximum area of bulbar conjunctiva. Then the area was illuminated from the temporal side and at approximately a right angle to the microscope objective. A binocular biobjective microscope was used to observe the details of the circulating blood and the reactions of the vessel walls at magnifications ranging between X32 and X216. The various components which were recognized were measured by use of a calibrated scale which was inserted into the ocular of the microscope.

Each period of examination was limited on the average to 15 minutes. During this period, serial observations were made, each lasting from one-half to two minutes with an equal period of rest between each observation. This procedure permitted study of acutely ill patients and produced minimum disturbance of the subject.

To obtain as objective data as possible which would permit comparison of the reactions observed with the microscope, answers to the following questions were obtained as the blood flowed through the arterioles, capillaries, and venules: Were cellular aggregates present or not? If so, were they in arterioles, capillaries, or venules, or any combination of these? The aggregates were composed of what: red cells, white cells, or platelets? If several kinds of aggregates were present, what was their ratio? What was the largest, smallest and average diameter of the aggregate in arterioles? In venules? What was the ratio of free cells to aggregates? How rigid were the aggregates? Did they resist passage through vessels, and how much? Did the aggregates plug vessels, and if so, for how long? What fraction of aggregates were rigid enough to plug? What was the frequency of replugging of arterioles? Did the aggregates fragment? If so, what was the site? What was the configuration of the aggregates? How was their configuration modified as they were forced through vessels smaller than they were? What was the appearance of the surface of the aggregates? What was the color of the aggregates? Were thrombi present? If so, what kind, red or hyaline?

In addition, the following information was sought in regard to the walls of the blood vessels and the parenchyma. What were the average diameters of arteries and venules? Were sacculations present? If so, what was their size? Was the permeability of venules altered? Were hemorrhages present? Were the distances between blood vessels which had blood flowing through them decreased or increased from normal? And, was there edema of the tissue?

Answers to the above questions were obtained and recorded on a special chart for each observation. At selected intervals the reactions were recorded by cinephotomicrography[5]. Subsequently, these data permitted a quantitative comparison of the extent and rate of change of the various components during the course of the disease.

Results. Two-hundred eighty patients were studied. Of this number, 205 acted as controls while 75 were patients with acute myocardial infarction. The 205 controls which were used in this study consisted of normal adults and patients with hypertensive arteriosclerotic heart disease with or without angina pectoris, inactive rheumatic heart disease with mitral stenosis and angina pectoris, and acute myocardial infarc-

*The patients with acute myocardial infarction came primarily from the service of Dr. Harold Feil, while the patients with structural heart disease came from the service of Dr. Claude S. Beck.

It is a pleasure to acknowledge the excellent cooperation of the staff and administration of the University Hospitals who made this study possible. The author is deeply grateful to Drs. Harold Feil, Claude S. Beck, and numerous other physicians who made it possible to study their patients.

tion prior to treatment with anticoagulants.

THE MICROSCOPIC REACTIONS OF THE BLOOD IN THE CONTROLS. A healthy circulation was found in healthy adults, in some patients with hypertensive heart disease with or without angina pectoris, and in some who had inactive rheumatic heart disease with angina pectoris. The microscopic appearance of the blood in these patients was as follows: The rate of flow in arterioles and venules was so rapid that no cellular detail was seen. In the capillaries, individual erythrocytes were seen. The walls of all vessels were smooth and the arterioles and venules were long tapering cones with the tip of the cone directed toward the capillary[19,21]. Healthy adults had no microscopically visible anemia or evidence of low blood volume (see[21]). However, the patients with hypertensive and structural heart disease usually showed some evidence of microscopically visible anemia in arterioles and an increase in the distance between open blood vessels.

Forty-five patients were examined who had structural heart lesions and who had been selected for cardiac surgery. Thirty-six of these patients were studied prior to cardiac surgery. It was found that in 19 the presence of static structural changes of the heart *per se* did not produce any change in the physical appearance in the circulation. In the others, erythrocyte aggregates measuring 10 to 13 micra were seen only in 20 to 30 micra venules.

Many of the controls were studied over a period of months. During this time it was not unusual for them to succumb to minor clinical syndromes like the common cold or exacerbations of seasonal allergies. These diseases caused the erythrocytes to aggregate, and such aggregates were seen only in venules smaller than 50 micra. In these vessels the aggregates measured between 12 and 20 micra. Such aggregates, however, were never seen in arterioles or in the larger venules. When these syndromes abated, so did the aggregates. These findings were in agreement with previous studies[2,15,19].

THE MICROSCOPIC REACTIONS OF THE BLOOD IN ACUTE MYOCARDIAL INFARCTION. Seventy-five patients with myocardial infarction were studied. The majority of these patients received Dicumarol alone, or heparin followed by Dicumarol. A small number of patients received Tromexan alone which was preceded by Paritol in a few cases (Table 1). The changes in the composition of the circulating blood and the blood vessel walls followed a pattern which varied only in degree of intensity and duration. When the subject had been in "good" physical condition prior to the first clinical episode of myocardial infarction, and the circulation was studied within the first 24 or 48 hours, it was not unusual to find the presence of a healthy circulation, healthy vessel walls, and no anemia. Twenty-four to 48 hours later, however, the intravascular and vascular picture had changed. The majority of the erythrocytes were stuck into aggregates, a small percentage of which (from 1 to 10%) intermittently plugged arterioles from a second to 20 seconds. Replugging of arterioles occurred repeatedly. Associated with these intravascular changes in arterioles were the rather marked changes in 15- to 50-micra venules. In these vessels the blood was a paste. The blood was dark red in color, the plasma spaces between cells and aggregates were lost, and the blood appeared like the packed cells in a hematocrit test. (*In vitro* examination of such blood in the electron microscope revealed a definite change of the surface of such cells. Figure 2, published elsewhere[4], contrasts the sur-

face appearance of such an erythrocyte with an erythrocyte obtained from the unagglutinated blood in health.) Concomitant with the intravascular changes were the changes in the integrity of the walls of the venules which became permeable to most of the plasma fluids and thus created a more than moderate edema of the surrounding tissue. In some patients the edema was so great that it was not possible optically to resolve the most superficial blood vessels. Multiple flask-shaped sacculations made their appearance slightly later, and less frequently isolated minute hemorrhages occurred through the walls of venules. Within the following day or two, multiple capillary or venous plugs (thrombi) of erythrocytes were noted. In the presence of this increase in the total viscosity of the blood, there was a further decrease in the velocity of blood flow. (Changes in the flow behavior of the blood were also present in the larger vessels as determined by *in vitro* rheology[6].) A generalized tortuosity and hypertrophy of venules accompanied the decrease in the velocity of flow. These changes were seen by the end of the second week. Usually within the next fortnight the majority of the intravascular and vascular changes receded. It was not unusual to see a healthy circulation (that is, unagglutinated blood) for a day or two which was then again replaced by a variable number of erythrocyte aggregates which plugged arterioles. In other patients a considerable degree of intravascular and vascular abnormality was maintained, and such patients were discharged with such lesions. Such a series of changes which have been described (with the exception of the last sentence) and wherein the circulation, the vessel walls, and the supporting tissue returned to normal, have been seen in nontransmural posterolateral infarction, but represent in a general manner the type of change which generally occurs in those patients with not too extensive cardiac damage.

The initial circulatory picture was different in those subjects who were more acutely ill on admission, in "shock." Here the initial microscopic picture revealed an avascular bulbar conjunctiva wherein all or most of the superficial blood vessels were shut. When a short segment of an arteriole was seen, erythrocyte aggregates were noted which were rigid and plugged these vessels for 30 seconds or longer and were then forced into dilated capillaries where they were stranded. The generalized constriction of arterioles was slow to recede (in those patients who survived the first 48 hours). For as long as 2 or 3 weeks thereafter, many arterioles remained shut, while others were constricted to one-half or more of their normal diameter. Associated with this constriction of arterioles was the generalized dilatation of venules. The normal ratio of arterioles to venules of 1:2 was replaced by ratios which varied between 1:4 to 1:6. The arteriolar constriction frequently preceded the appearance of erythrocyte aggregates of sufficient rigidity to interfere with the blood flow in these vessels.

One of the most significant and consistent findings in this study was the relationship of the anticoagulant, the prothrombin concentration, erythrocyte aggregation, and the reaction of the walls of the venules. In general, irrespective of the anticoagulant used and the prothrombin concentration, whether 10 or 30%, erythrocyte aggregates were often present of such a size and rigidity to significantly resist passage or plug arterioles, or both, for varying periods of time, from 2 to 30 seconds, as well as being stranded in venules (see Tables 3 to 5). Therefore, it was con-

cluded that a low prothrombin concentration was no indicator of the effectiveness of the anticoagulant in preventing microthromboembolism or in maintaining a desirable fluidity of the blood. Further, fluctuations in the prothrombin concentration had an equivocal or slight effect on the disintegration of the erythrocyte aggregates. The physical character of the erythrocyte aggregates was not appreciably changed when the prothrombin fluctuated between 10 and 40% of normal.

Hyaline or leukocyte aggregates were seen occasionally in some patients, but in no patient in this series were such aggregates present in sufficient number during any period as to interfere seriously with the general blood flow. When hyaline aggregates were seen, they were usually limited to a segment of a venule where they partially occluded the lumen. The largest hyaline aggregates which were seen measured 60 micra in length and 30 to 40 micra in width. The largest hyaline aggregates were seen only in patients who received anticoagulant therapy.

The abnormality of the walls of venules was often increased by the anticoagulant above that produced by the retardation of flow produced by the aggregates. The most significant change was a loss in the "tone" of venules which resulted in flask-shaped sacculations which often appeared on the "point of rupture." Extravasation of erythrocytes and localized flame-shaped hemorrhages did occur through the walls of such venules. These changes were produced under Dicumarol and Tromexan therapy, and the lesion was always limited to venules measuring less than 100 micra in diameter.

Specific illustrations of these intravascular reactions are cited in Tables 3 to 5. They illustrate the typical reactions in cases of "simple" and "complicated" myocardial infarction. In a third of the cases the erythrocyte aggregation improved or was entirely eliminated following anticoagulant therapy, but in 44% the aggregates were not influenced, or the physical characteristics became worse (larger and more rigid), this occurring in 21% of this group. These results are tabulated in Tables 1 and 2 in regard to age, sex, and type of treatment.

Case Reports. CASE CB (Table 3) illustrates the intravascular reactions which follow an acute myocardial infarct.

The patient was a 45-year-old white male who was admitted with chest pain of 8 hours' duration. He had been active and asymptomatic until 2 months ago when he began to experience pressing substernal pain on exertion which radiated to the upper left arm. On the day of admission the pain began without exertion and was not relieved with nitroglycerin. The physical findings were within the normal physiologic range. The electrocardiogram prior to admission had been equivocal, but on admission the changes in its pattern were compatible with a recent nontransmural posterolateral myocardial infarct which was substantiated by later electrocardiograms. The clinical impression and electrocardiographic evidence established the diagnosis of an acute infarction of the myocardium due to arteriosclerotic coronary thrombosis. The patient was treated with Dicumarol, and his hospital course was uneventful.

COMMENT. The prothrombin concentration was 40% or more during the first 5 hospital days and was not adequately controlled until the tenth day. The circulation was essentially healthy on the time of admission and reverted to this state by the 22nd hospital day. On re-admission 5 months later for severe substernal pain, the electrocardiogram did not reveal any evidence of a new infarct, and 9 months later when elective surgery was done (at this time there were no symptoms referable to the cardiovascular system) the electrocardiogram was within normal limits.

CASE LH (Table 4) illustrates the maintained presence of intravascular erythrocyte

aggregates and vessel wall pathology in the presence of low prothrombin values. The pathologic process was inadequately controlled which resulted in repeated infarctions.

On the first admission of this 64-year-old white female, the chief complaint was "pressing" pain in the chest of 2 hours' duration. Similar pain after exertion had occurred 11 days earlier when the pain lasted 15 minutes and was controlled with nitroglycerin. This pain recurred 6 days before admission. Three days prior to admission an electrocardiogram revealed evidence of subendocardial ischemia. On the day of admission similar pain recurred which was not relieved by nitroglycerin.

TABLE 1.—AGE DISTRIBUTION OF PATIENTS AND COURSE OF ERYTHROCTYE AGGREGATION IN MYOCARDIAL INFRACTION

	Erythrocyte Aggregation				*Sex*	
Age (years)	*Improved*	*Unimproved*	*	*Total*	*M*	*F*
30–39	0	2	..	2	2	..
40–49	6	6	1	13	12	1
50–59	9	7	3	19	15	4
60–69	7	11	9	27	16	11
70–79	3	7	3	13	6	7
80–89	1	0	..	1	1	..
Total	25	33	16	75	52	23

* Either died within the first forty-eight hours or multiple observations were not possible

TABLE 2.—THE EFFECT OF ANTICOAGULANT THERAPY ON ERYTHROCYTE AGGREGATION IN MYOCARDIAL INFARCTION

	Erythrocyte Aggregation			
Treatment	*Improved*	*Unimproved*	*Significant Increase*	*Healthy Circulation (re-established)*
Dicumarol	15	26	6	8
Tromexan	6	3	1	2
None	4	4	0	0
Total	25	33	7	10

Physical examination on admission revealed: Temperature, 36.5° C., pulse 84/minute, respiration 16/minute and a blood pressure of 170/100. The patient was moderately obese. There was no dyspnea or cyanosis. No other pertinent physical findings existed.

The significant laboratory data were, an erythrocyte count of 5.3 × 10^6/c.mm., white cell count of 7,000/c.mm., a sedimentation rate of 18 mm./hr., and a glucose tolerance curve which rose to 222 mg./100 cc. in 2 hours and continued to be elevated in the fourth hour (133 mg.) with a glycosuria of two plus. The electrocardiogram indicated a left axis deviation with an anterior infarct and subsequent electrocardiograms showed an evolvement from a nontransmural infarct to a transmural infarct.

The first hospital course was uneventful. The patient was maintained on Dicumarol. Ambulation was begun on the 11th day. For several days after admission the patient had substernal pain which was relieved with morphine and Demerol. The lowest blood pressure was 130/70, and the highest 160/110. The diabetes was controlled with diet.

From the clinical and laboratory data the diagnosis was infarction of the myocardium due to arteriosclerotic thrombosis; arteriosclerotic heart disease; and diabetes mellitus.

This patient was re-admitted 18 days later with a new myocardial infarct. During this admission she was treated with heparin and Dicumarol.

CASE GL (Table 5) illustrates the intravascular and vascular reactions which probably occur from multiple sources of tissue destruction.

This 47-year-old white female was a known hypertensive of 15 years' duration with an average blood pressure of 250/150. Three years ago she was hospitalized for 6 months because of apoplexy, which resulted in left-sided weakness and numbness. For the past

TABLE 3.—SIGNIFICANT DATA IN CASE CB

Hospital Day	*Duration of Plugging of Arterioles by Erythrocyte Aggregates*	*Size of Erythrocyte Aggregates† in Arterioles*	*Degree of Reduction of Flow in Arterioles**	*Size of Erythrocyte Aggregates† in Capillaries*	*Size of Erythrocyte Aggregates† in Venules*	*Ratio of the Diameter of Arterioles to Venules*	*Prothrombin Concentration in Per Cent*	*Remarks*
1	0	8μ	None	0	10–13μ	(Normal)		Sed. rate 6
						1:2	100%	Hemato. 52
								RBC 6
5	1 second	10—13 × 13 18μ	Moderate to severe	13—16μ 13—16μ	13—18μ 13—18μ	1:3	40	Occ. hemorrhages Hypertrophy of venules +++
11	0	10—13μ	Moderate to severe	..	..	1—3:4	18	Sed. rate . . . . 6 Hemato. . . . . 51 Occ. hemorrhages Hypertrophy of venules +++
15	0	8—10μ	None	0	10—13μ	1:2	24	.. ..
22	0	8μ	None	0	10μ	1:2	26	.. ..

* None = flow too fast to see either cells or aggregates
Moderate = flow of cells or aggregates easily visible
Severe = flow cells or aggregates barely moving

† The figures in these columns indicate the size of erythrocytes or aggregates. 8μ means that only individual erythrocytes are present; 10—13 × 13—18μ means that all erythrocytes are stuck into aggregates which vary in size as indicated.

Sed. rate = sedimentation rate Hemato. = Hematocrit RBC = red cell count in millions per cmm.

TABLE 4.—SIGNIFICANT DATA IN CASE LH

Duration of Plugging of	*Size of*	*Degree of*	*Size of*	*Ratio of the*

TABLE 4.—SIGNIFICANT DATA IN CASE LH

Hospital Day	*Duration of Plugging of Arterioles by Erythrocyte Aggregates*	*Size of Erythrocyte Aggregate in Arterioles*	*Degree of Reduction of Flow in Arterioles*	*Size of Erythrocyte Aggregates in Venules*	*Ratio of the Diameter of Arterioles to Venules*	*Prothrombin Concentration in Per Cent*	*Remarks*
1	Frac. sec. to 30 secs.	13 — 78μ	Moderate to severe	Multiple capillary and venous red cell thrombi	1:4—5	100	RBC . . . . . 5.5 Sed. rate . . . 18 Hemato. 40
3	1 to 4 secs.	10 — 106μ	Severe	No change	..	28	(Multiple sacc.)
5	1 to 2 secs.	10 — 78μ	Severe	Multiple red cell thrombi	1:5	32	RBC . . . . . 5.37 Sed. rate . . . 41 Hemato. 43
7	1 to 5 secs.	13 — 150μ	Severe	Multiple red cell thrombi	1:3	23	..
11	1 sec.	10 — 13μ	Moderate	Occ. thrombus Agg. 30—40μ	1:3	15	..
15	1 sec. 0	13 — 26μ (10%) 10μ (90%)	Moderate None	Multiple capillary and venous red cell thrombi Agg. 20—70μ	1:3	34	..
16	0	10μ (90%)	None	Multiple capillary and venous red cell thrombi Agg. 20—70μ	1:3	26	..
20	1 sec. 0	16 — 52μ (10%) 10μ (90%)	Severe None	Multiple capillary and venous red cell thrombi	1:2	19	..
24	0	10μ (90%)	None	Multiple capillary and venous red cell thrombi	1:2	84	..
28	0	8 — 10μ	None to slight	No thrombi	1:2	100	..
44*	1 to 30 secs.	10 — 78μ	Severe	Multiple capillary and venous red cell thrombi	1:4	87	..
49	1 to ? secs.	10 — 78μ	Severe	Multiple red cell thrombi, plasma spaces 150μ between aggregates	..	22	..
56	1 to 30 secs.	8 — 78μ	None to severe	Multiple red cell thrombi in capillaries, 150μ aggregates in venules	..	13	Sed. rate . . . 32 Hemato. 41
57	1 to 10 secs.	8 — 40μ	None to severe	Reduction in thrombi	..	16	..

* First day or re-admission Sed. rate = sedimentation rate Hemato. = hematocrit

2 years there have been approximately 6 episodes in which the patient developed first a generalized erythematous eruption, followed within 15 minutes by weakness and numbness which was limited to the face and later became generalized. The duration of such an episode was one to 3 hours. There were no other significant abnormalities in the history.

On the evening of admission the patient had a similar episode as described above with the addition of a period of unconsciousness lasting several minutes. Physical examination revealed the following: A well-developed, well-nourished white female who appeared stuporous and uncooperative, able to answer questions but only after intensive stimulation. The temperature was 36.3° C., pulse 50/minute, respiration 16/minute, and a blood pressure of 158/108. The skin revealed fine erythematous eruptions on the thorax and upper extremities. The heart was enlarged to the left, and the rhythm was slow and regular; murmurs were absent. There was a two plus edema of the lower extremities.

A routine electrocardiogram shortly after admission revealed a recent posterior myocardial infarct with anterior involvement. The patient was given Dicumarol. An electroencephalogram showed a basic 8 per second alpha rhythm which was interpreted as a "borderline" record.

Forty-eight hours after admission the patient became rational, and there was found to be weakness of the left side. Rapid clinical improvement became evident by the third day which was maintained until the time of discharge.

The final clinical impression was: recent posterior myocardial infarct with anterior involvement; cerebral encephalomalacia due to arteriosclerosis and remote thromboses; hypertensive cardiovascular disease; cardiac enlargement and insufficiency.

Discussion. This study introduces a new factor for consideration in the pathogenesis of acute myocardial infarction in man: erythrocyte aggregation. The aggregates alter the physical composition of the blood, making it more difficult to pump such blood through the vessels. *In vivo* microscopic observation demonstrated that the aggregates resist passage to a significant degree through vessels measuring less than 50 micra in diameter.

Erythrocyte aggregates have been described frequently in the literature[7-9, 11,12,15-17,23-25,27,28,34,37,38], but critical descriptions or analysis of this reaction were not made. Prior to about 1933, it had not been possible to define extensively the composition of the blood flowing in internal organs at a microscopic level. The ability to know what to look for and how to define these reactions in health and contrast them with those occurring in disease occurred for the first time between 1940 and 1950[3,20,21]. The majority of papers even now fail to define, first, whether the intravascular reactions occur in the arterial or in the venous circulation, and in addition (and equally important) the major physical characteristics of the aggregates[25,37,42]. It is of considerable importance to differentiate clearly those reactions occurring in the arterial blood from those occurring in capillary or venous blood. The arterial aggregates represent a statistically valid sample of all of the arterial blood, while those in the capillary or venous blood may primarily be due to local pathology.

To begin to understand the role that erythrocyte aggregation may play in the pathogenesis of myocardial infarction, it is necessary to review in general the theory and facts of their formation, their relation with the clotting mechanism of blood and anticoagulants.

The current theory[1,3] which has been proposed for the production of erythrocyte aggregates is as follows: Phase I, the release of substances from damaged tissue cells into the circulation; phase II, reaction of these substances with the plasma to form precipitates which in phase III coat erythrocytes causing them to stick to each other (the leukocytes and platelets are perhaps similarly affected). And in phase IV there is an interaction of the coating with the surfaces of erythrocytes

TABLE 5.—SIGNIFICANT DATA IN CASE GL

Hospital Day	*Duration of Plugging of Arterioles by Erythrocyte Aggregates*	*Size of Erythrocyte Aggregates in Arterioles*	*Degree of Reduction of Flow in Arterioles*	*Size of Erythrocyte Aggregates in Capillaries*	*Size of Erythrocyte Aggregates in Venules*	*Ratio of the Diameter of Arterioles to Venules*	*Prothrombin Concentration in Per Cent*
1	1 to 2 secs.	13—16μ (25%)*	Severe	Multiple erythrocyte thrombi	13 × 26—50μ	1:5	No anticoagulant
	0	10μ (75%)	None				
2	30 to 60 secs.	16 × 26μ (50%)	Severe	Multiple erythrocyte thrombi	20 × 50μ	1:5	..
	0	13 — 16μ (50%)	Moderate				
4	30 to 60 secs.	16 × 26μ (50%)	Severe	Multiple erythrocyte thrombi	20 × 50μ	1:5	20
	0	13 — 16μ (50%)	Moderate				
6	1 to 2 secs.	13 × 16 — 25μ	Moderate Severe	Decrease in erythrocyte thrombi	20 × 50μ	1:5	19
8	Frac. sec.	10 × 52μ (20%)	Severe	(Most capillaries shut)	13—50μ	1:5	20
	0	10μ	None				
10	Frac. sec.	10 × 52μ (20%)	Severe	Erythrocyte thrombi multiple	13—26—30μ	1:5	20
	0	10μ	None				
12	1 to 2 secs.	13 — 20 × 16 — 30μ (10%)	Severe	Erythrocyte thrombi decrease	13—26μ	1:4	25
	0	10μ (90%)	None None				
18	Frac. sec. to 1 sec.	10 — 13 × 26 — 52μ (Most this size)	Severe	No erythrocyte thrombi	..	1:4—7	19
20	Frac. sec. to 1 sec.	10 — 13 × 26 — 52μ (Most this size)	Severe	No erythrocyte thrombi	..	1:4—7	19

* The percentage estimates are approximate and are meant to indicate that there were two different kinds of aggregates in arterioles.

which determines whether or not the coatings remain adherent to the surfaces.

There are several kinds of evidence which indicate that injury to tissue results in erythrocyte aggregation[2,3,19,20]. The injurious agent may be physical or chemical. An example of the former is mechanical trauma. When the technique of microtrauma is used it is possible to study the control and experiment simultaneously in one microscope field. By direct visualization it is possible to direct the traumatizing instrument to a selected portion of the field and thereby reserve the remainder for the control. When the control is healthy, then *all* the erythrocytes flowing through the various blood vessels are seen as *single* cells. This is in sharp contrast to what is seen immediately *after* crushing part of the field. In less than one second after inflicting the crushing injury, *all* the erythrocytes are clumped in the capillaries and venules in the crushed area. The size of aggregates varies from three to ten times the diameter of one erythrocyte. In the nontraumatized areas the erythrocytes remain single. Similar results are observed when crushed tissue like brain or muscle from a donor animal is placed on a tissue like mesentery in a test animal. An example of the second is that produced by disease. Here there is a considerable variation in the intensity and duration of the reaction[3,21]. Bacterial diseases like pneumococcus pneumonia and protozoan diseases like malaria are very effective in rapidly involving the majority of the host's erythrocytes in aggregates which are large and rigid. Other diseases like the common cold or the disease syndrome referred to as atypical pneumonia produce either minimal erythrocyte aggregation or the aggregates are very small or plastic, or both.

The above indicate that some substance or substances of unknown composition are released following injury to a tissue which results in erythrocyte aggregation. There is some evidence to indicate that at least under certain conditions, these substances are related to the clotting mechanism of the blood[35,39]. This evidence comes from varied sources. In the living animal clear, highly refractile, hyaline substances, with or without streamers, have been seen encompassing the erythrocyte aggregates. When such aggregates were examined with the dark field microscope, they were seen to be encompassed by highly refractile coatings. And, when such coatings were microdissected, tenacious threads were withdrawn[20]. The physical appearance of these coatings was suggestive of fibrin. Furthermore, chemical tests indicated that the substance was either fibrin or fibrin-like[1]. This possibility was tested in rhesus monkeys infected with malaria[20]. In this disease one of the major causes of death is due to the formation of large, rigid erythrocyte aggregates. When heparin was given early in the disease, the large and rigid aggregates did *not* form. The fluidity of the blood was kept within normal limits.

Malaria presents an ideal disease to test the ability of an anticoagulant to prevent or modify erythrocyte aggregation. In this disease the invading organism is in the blood, which makes it possible to obtain rapid and intimate contact with the diseased tissue responsible for releasing the substances into the blood stream which cause the cells to clump. The results of anticoagulant therapy in malaria indicated that this form of therapy can be effective in preventing or significantly reducing erythrocyte aggregation under *optimal* conditions.

The role of erythrocyte aggregation in the pathogenesis of acute myocardial infarction may now be examined. The

pattern of production of the aggregates is suggestive that they are produced by the release of substances from hypoxic or degenerating cardiac muscle, or both. The basis of this statement rests upon the evidence obtained from those patients who had a circulation which was free of such aggregates on admission. After a few days the erythrocytes clumped and were of sufficient rigidity to resist passage in arterioles and often plugged them. No other cellular elements, (for example, leukocytes or platelets) played any significant part in reducing the linear velocity of flow other than at the site of injury. The intravascular changes waned or disappeared as clinical improvement occurred. It would be anticipated that the site, extent, and rate of the degenerating cardiac muscle would determine the degree and duration of the erythrocyte aggregation.

The question arises: How do the anticoagulants affect the blood? The numerous clinical studies which have been made on the effect of anticoagulant therapy on acute myocardial infarction leaves little doubt that these drugs favorably influence the course of this disease in many patients[14,26,41]. It does not necessarily follow, however, that the anticoagulants consistently revert the systemic blood to its normal fluidity. They do not. It is to be anticipated when the majority of the vessels are thrombosed there will be little or no release of substances into the systemic circulation to cause erythrocyte aggregation. Conversely, many aggregates will form when the circulation is maintained through the injured area. Further, it is not impossible to conceive that an insufficient concentration of anticoagulant would be present locally, irrespective of the systemic prothrombin level, when large volumes of substances are intermittently released which cause agglutination. This hypothesis may explain the presence of erythrocyte aggregation in the presence of clinically effective concentrations of anticoagulants.

Several points need clarification. One is that the presence of erythrocyte aggregates *per se* is only indicative of tissue destruction. The aggregates are secondary; that is, they occur *after* the *initial* injury. Therefore, they are not pathognomonic for acute myocardial infarction. Neither is it possible at the present to state the extent of damage produced by the erythrocyte aggregates. It will not be possible objectively to assay the damage that this kind of abnormality does to the host until the disease can be re-studied in their absence. And it must be re-emphasized that the intravascular reactions seen in the *arterioles* of the bulbar conjunctiva represent a statistically valid sample of the systemic arterial blood. The reactions seen to occur in the capillaries and venules of the bulbar conjunctiva may not necessarily represent a statistically valid sample. This is especially true in respect to the intravascular and vascular reactions which are anticipated to occur in the capillaries and venules of the injured cardiac muscle, which will be considerably greater than in any other part of the vascular system.

The other objectives of the investigation were not attained. This study was unable to answer the question of the length of time that anticoagulant therapy should be maintained until erythrocyte aggregation is removed. Neither was it possible to give an opinion about the optimal concentration of anticoagulant for a particular case. The results of this study did indicate, however, that the maximum safe concentration of anticoagulant which can be given to a patient is

usually inadequate in preventing microthromboembolism. Therefore, it is suggested that other drugs should be considered which will prevent erythrocyte aggregation without interfering with the clotting mechanism of the blood.

Summary. An *in vivo* microscopic test is introduced to determine the fluidity of the systemic blood in acute myocardial infarction.

In acute myocardial infarction erythrocyte aggregates were found which produced intermittent embolization of arterioles; such aggregates were not found in the control group.

The prothrombin concentration was found to have an inconstant relationship to the fluidity of the blood. In the presence of prothrombin concentrations between 10 and 30%, erythrocyte aggregates were present which seriously reduced the linear velocity of the blood flow or plugged arterioles. The prothrombin concentration was considered an unreliable indicator of the fluidity of the blood.

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POSTMORTEM STUDIES ON CORONARY ATHEROSCLEROSIS, SERUM BETA LIPOPROTEINS AND SOMATOTYPES*

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The relationship and the significance between serum beta lipoproteins and the degree of atherosclerosis is still unsettled. There is considerable agreement concerning the more frequent association of certain body types with atherosclerosis[3,5]. This investigation was undertaken to determine by more objective observations the interrelationships of the serum beta lipoproteins, the somatotypes, and the degree of coronary and aortic atherosclerosis.

One hundred and fifty-seven consecutive, apparently healthy individuals, who had died suddenly from violence (accident, suicide and homicide) or suddenly from coronary occlusion, were somatotyped according to a previously described modification of Sheldon's methods[5]. In these deceased individuals postmortem examination revealed no other significant lesions or evidence of disease aside from the traumatic ones and the coronary arterial lesions. Cases with evidence of chronic or other significant diseases were not included in this study because serum beta-lipoproteins may be temporarily altered in conditions other than atherosclerosis. These deceased individuals were necropsied and studied with particular reference to the degree of aortic and coronary atherosclerosis. Since the degree of coronary atherosclerosis paralleled that found in the aorta quite closely, reference will be made only to the lesions in the coronary arteries. The technique for the grading and examination of the coronary arteries was similar to that used in a preceding study which included injection of the coronary arteries with radio-opaque material[5]. Prior to postmortem examination, blood was withdrawn from all chambers of the heart by means of a long sterile needle and syringe. Crystals of merthiolate were added to the separated serum which was kept under refrigeration until the beta-lipoproteins were determined by means of ultracentrifugation according to the technique of Gofman[4]. The serum beta-lipoprotein determinations were performed as a blind study and only related to the other findings after these had been determined and recorded.

The validity of postmortem serum beta lipoprotein patterns was ascertained by obtaining postmortem blood samples from deceased patients upon whom similar determinations had been made within 3 hours prior to death

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(see Table 1). The variations between the pre- and postmortem values were within the range of expected laboratory error. Furthermore, comparative studies were made on blood samples taken both 3 and 20 hours postmortem on 5 cases to determine the rate of postmortem alteration in the serum beta-lipoprotein levels. No significant alteration was noted within a 20-hour postmortem period. Most of the bloods studied were obtained within 6 hours after death.

One hundred and forty-three cases were then classified into four groups (see Table 5). The other 14 cases were separated because of postmortem evidence of recent myocardial infarction. It is known that in the presence of recent myocardial infarction there may be temporary alteration in the serum beta-lipoprotein patterns[3]. Findings in the 125 cases concerning the correlation of the degree of atherosclerosis with abnormalities in the lipoprotein pattern may be observed in Table 5.

TABLE 1.—ANTEMORTEM SERUM LIPOPROTEINS COMPARED WITH POSTMORTEM SERUM LIPOPROTEINS*

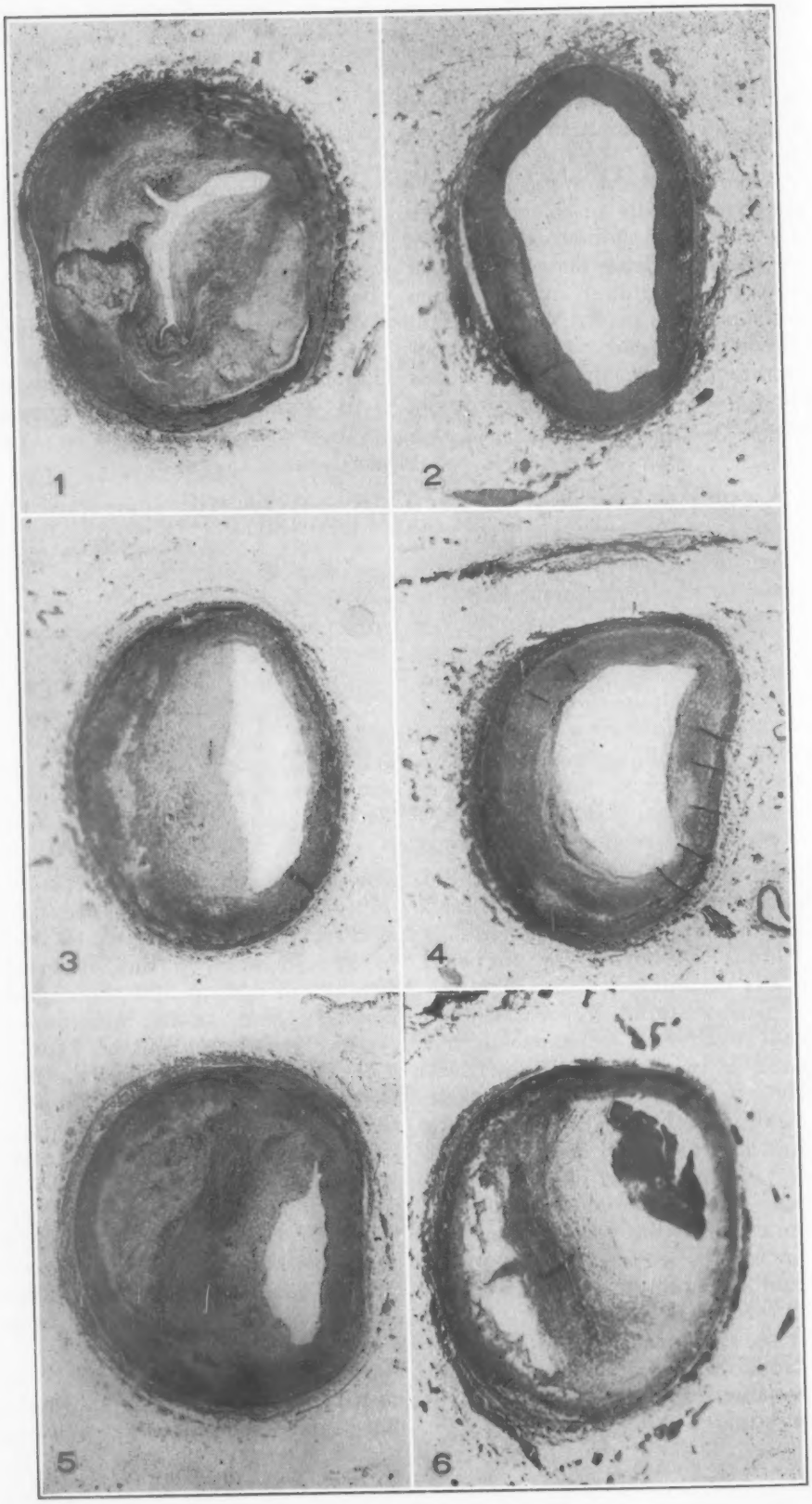
Specimen	*Sf 12–20*	*Sf 20–100*
1351	68	100
1351 (a)	70	94
1427	88	73
1427 (a)	82	76
A6	41	46
A6 (a)	37	39
58	23	34
58 (a)	24	34
30	104	100
30 (a)	109	97

* (a) Postmortem blood sample.

Based upon previous clinical and laboratory studies a table of "normal" values for serum beta-lipoproteins at the various age levels and for both sexes was established (see Table 2)[4]. The "normal" table based on 1000 clinically evaluated individuals is not regarded as being finite. It serves as an arbitrary figure for the purposes of this study. Further investigation may require alteration of these "normal values." In previous[5] as well as in concomitant studies on similar groups of necropsied cases the patterns and degree of atherosclerosis for the various ages and somatotypes were also determined (see Tables 3 and 4). These served as arbitrarily established base line standards for the classification and interpretation of the findings in the present study.

One hundred and twenty (84%) of the 143 cases revealed a positive correlation regarding the presence or absence of abnormalities in the serum beta-lipoprotein pattern and the degree of coronary and aortic atherosclerosis consistent with age and sex (see Figs. 1 to 5). There was a lack of correlation in 23 (16%) of the 143 cases. Of these 23 cases, 11 revealed a "normal" serum beta-lipoprotein pattern and an advanced degree of coronary and aortic atherosclerosis for the respective ages. In the other 12 there was an "abnormal" serum beta lipoprotein pattern associated with insignificant amounts of coronary and aortic atherosclerosis.

Further subdivision of these cases into the various somatotypes revealed that in the ectomorphic group of males



Figs. 1–6. Legend on opposite page.

TABLE 2.—"NORMAL" VALUES OF SERUM BETA-LIPOPROTEIN (Sf 12–20 and 20–100 Classes) AT VARIOUS AGE LEVELS

Age in Years	*Sf 12–20*	*Sf 20–100*
0–14	0–14	6–30
15–30	15–30	30–50
30–40	30–40	30–60
40–50	30–40	40–70
50–60	30–40	40–70
60–70	30–40	40–70
75 plus	30–40	40–70

TABLE 3.—TYPE OF PHYSIQUE AND THE DEGREE OF CORONARY SCLEROSIS

(100 Males under 46, Victims of Accidental Death)

Body Type	*Number of Cases*	*Average Age*	*Degree of Coronary Sclerosis* 0	1+	2+	3+	4+
Mesomorph	39	36	4	10	14	7	4
Ectomorph	31	35	17	8	5	1	0
Endomorph	13	35	4	7	2	0	0
Mixed	17	34	6	6	3	2	0
Total	100		31	31	24	10	4

TABLE 4.—DISTRIBUTION OF SOMATOTYPES IN 64 CONSECUTIVE NECROPSIED CASES OF SUDDEN DEATH FROM CORONARY OCCLUSION IN MALES UNDER 46

(Including Cases from Present and a Previous Study)

Dominant Somatotype	*Number of Cases*
Mesomorph	44
Ectomorph	6
Endomorph	3
Mixed	13
Total	66

Fig. 1.—Photomicrograph of coronary artery with advanced sclerosis in a thin, normotensive, nondiabetic female, aged 37, who died suddenly from acute coronary insufficiency. Serum beta-lipoproteins: S_f 12–20 was 66 and S_f 20–100 was 90 ("abnormal" values).

Fig. 2.—Photomicrograph of coronary artery with no sclerosis in an apparently healthy male, aged 24 (mixed somatotyped), who died suddenly in a traffic accident. Serum beta-lipoproteins: S_f 12–20 was 16 and S_f 20–100 was 30 ("normal" values).

Fig. 3.—Photomicrograph of coronary artery with marked sclerosis in an apparently healthy young male, aged 22 (mesomorph), who died suddenly in an industrial accident. Serum beta-lipoprotein: S_f 12–20 was 50 and S_f 20–100 was 63 ("abnormal" values).

Fig. 4.—Photomicrograph of coronary artery with minimal sclerosis in an apparently healthy male, aged 45, who died suddenly in a traffic accident. Serum beta-lipoproteins: S_f 12–20 was 26 and S_f 20–100 was 37 ("normal" values).

Fig. 5.—Photomicrograph of coronary artery with advanced sclerosis in a 42-year-old male (mesomorph) who died suddenly from coronary occlusion. Serum beta-lipoprotein: S_f 12–20 was 140 and S_f 20–100 was 160 ("abnormal" values).

Fig. 6.—Photomicrograph of coronary artery with advanced sclerosis of a 39-year-old male (ectomorph) who died suddenly from coronary occlusion. Serum beta-lipoproteins: S_f 12–20 was 27 and S_f 20–100 was 21 ("normal" values).

TABLE 5.—CORRELATION OF DEGREE OF CORONARY SCLEROSIS WITH SERUM BETA-LIPOPROTEIN IN 143 CONSECUTIVE NECROPSIES

(Fourteen Cases with Recent Myocardial Infarction Are Excluded)

	Under 20	*21–30*	*31–40*	*41–50*	*51–60*	*61–70*	*Over 70*	*Total*	
Average degree of sclerosis for age and sex. "Normal" serum beta-lipoprotein.	3	6	3	11	7	5	5	40	120 (84%) positive correlation
Advanced degrees of sclerosis for age and sex. "Abnormal" beta-lipoprotein.	1	8	14	29	22	6	0	80	
Average degree of sclerosis for age and sex. "Abnormal" serum beta-lipoprotein.	0	1	1	5	4	1	0	12	23 (16%) no correlation
Advanced degree of sclerosis for age and sex. "Normal" serum beta-lipoprotein.	1	0	1	6	2	1	0	11	

(see Fig. 6) there was the least degree of positive correlation (see Table 6). In the ectomorphic males, 11 out of 28 cases failed to reveal a positive correlation, whereas in the remaining group of males (endomorphs, mesomorphs and mixed groups) only 11 out of 105 failed to correlate in a positive manner. Only one of the 13 females revealed a discrepancy between the serum beta-lipoprotein pattern and the degree of atherosclerosis.

TABLE 6.—CORRELATION OF DEGREE OF CORONARY SCLEROSIS WITH SERUM BETA-LIPOPROTEIN

	Sex and Body Type		
	Number Cases	*No Correlation*	*Correlation*
Males	133	..	..
Ectomorphs	28	11	17 (60%)
Mesomorphs, Endomorphs, mixed	105	11	94 (90%)
Females	13	1	12 (92%)

TABLE 7.—SERUM BETA-LIPOPROTEINS IN PATIENTS WHO DIED SUDDENLY FROM CORONARY OCCLUSION

	Advances Coronary Sclerosis. No Thrombus or Recent Infarct	*Advanced Sclerosis with Thrombus and Recent Infarct*	*Totals*
"Abnormal" Serum beta-lipoprotein	52	6	58
"Normal" Serum beta-lipoprotein	6	9	15
Total	58	15	73

Seventy-three of the 157 cases died suddenly from coronary insufficiency. In 15 of these 73 cases, coronary thrombosis with recent myocardial infarction was present. In the others (58 cases) the only significant findings consisted of advanced atherosclerosis of the coronary arteries with varying degrees of stenosis. Fifty-two of these latter 58 cases revealed "abnormal" serum beta-lipoprotein patterns, whereas only 6 of the 15 with myocardial infarcts showed an "abnormal" pattern (see Table 7).

Discussion. Practically all previous studies concerning the relationship of serum beta-lipoproteins to human atherosclerosis have been based upon clinical evaluation during life. The problem of adequate "normal" controls, in the form of individuals considered to have no evidence of coronary atherosclerosis, has made evaluation of the data difficult. Included among the "normal" controls were undoubtedly persons with variable and advanced degrees of coronary and aortic atherosclerosis, in whom no clinical manifestations of coronary insufficiency were present. Relative to this, studies on the correlation of ballistocardiograph patterns with changes in the spectrum of the serum beta-lipoprotein have recently yielded promising results[1]. Numerous studies indicate that in many apparently healthy young

males there exist significantly advanced degrees of coronary and aortic atherosclerosis[3,5]. On the other hand, clinical evidence of coronary insufficiency does not necessarily imply the presence of advanced degrees of atherosclerosis, for it is known that a thrombus may be superimposed upon a very small atherosclerotic plaque. An abnormal pattern of the coronary arterial tree with hypoplasia of one of the major branches may result in an insufficient supply of blood to the myocardium. The lumen of the coronary artery may be narrowed by an inflammatory lesion. A quantitatively minor degree of atherosclerosis may be strategically located in the form of an isolated plaque and partially or completely occlude the lumen. These facts have led to difficulties in the proper evaluation of the relationship between serum beta-lipoproteins and coronary artery atherosclerosis.

In this study these obstacles were eliminated because the degree and distribution of the atherosclerosis in both the coronary arteries and aorta were measured directly at necropsy. However, there were several other limitations inherent in the nature of the study. Only one serum beta lipoprotein determination was available on each case, so that the previous beta-lipoprotein level or the duration of the present one was unknown. For instance in an adult who on postmortem examination revealed an abnormal beta-lipoprotein level and in whom no coronary or aortic atherosclerosis was observed, it was entirely possible that this could have been the very inception of the atherosclerotic process. In this individual perhaps a few more years of an altered beta-lipoprotein level would eventually have been associated with more advanced degrees of sclerosis. In older individuals with "normal" levels and "abnormal" coronary arteries it was possible that in previous years there might have been abnormal beta lipoprotein levels present over sustained periods of time. It was also obviously impossible to dissect and study the arterial tree of the extremities. This of couse would have been the ideal. In view of these limitations it is indeed surprising and significant that such a close association was obtained. The greatest discrepancy was observed in the ectomorphic males. This low degree of correlation in the ectomorph would at this time at least preclude any conclusions concerning the cause and effect relationship of serum beta-lipoprotein to the development of atherosclerosis. The differences in degree of correlation between the ectomorphic males and the other groups are provocative and require further investigations. Studies concerning the endocrine make-up of these somatotypes may eventually help to explain these differences. Investigation along these lines is in progress. There have been previous reports stating that in diabetes mellitus abnormalities in the serum beta-lipoprotein pattern are related to the presence of "nephropathy" rather than to the amount of atherosclerosis[2]. The present study would tend to negate this claim since the postmortem observation of the kidneys revealed no anatomic evidence of those changes specifically known to be associated with diabetes mellitus.

Evidence would indicate that any male or female with the exception of the ectomorphic male who has an abnormal serum beta-lipoprotein pattern must be seriously regarded as a potential victim of atherosclerosis. This is particularly true of the mesomorphic male.

Summary. 1. Serum beta-lipoprotein patterns, the dominant somatotype and the degree of aortic and coronary

atherosclerosis were determined at necropsy on 157 individuals who had died suddenly from violence or natural causes.

2. There was an over-all correlation of 84% between the serum beta-lipoprotein pattern and the degree of aortic and coronary atherosclerosis. This correlation is highly significant ($p<0.1$).

3. The ectomorphic male proved to be an exception in that there was 60% positive correlation, whereas in the other groups exclusive of the ectomorphs there was a 90 to 92% correlation.

4. These studies offer further confirmation of the greater degree of atherosclerosis in the mesomorphic male than in the ectomorphic male.

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DERMATOPHYTES OF ANIMAL ORIGIN TRANSMISSIBLE TO MAN

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"It is one of the most interesting questions for man to know what are the diseases which he can contract from the animals which surround him. It is very interesting to study also in a thorough way what cutaneous diseases can pass from animals to man."
—Raymond Sabouraud, 1908.

THE dermatophytes, species of the genera *Epidermophyton*, *Microsporum*, and *Trichophyton*, form a homogenous group of specialized fungi. From the patho-physiological point of view, they are characterized by their highly developed parasitism and host-specificity; they affect only the keratinized layers of the skin, nails and hairs of man and animals[28,37,49,50,51,55,78,203].

The dermatophytes invade only the living keratin. In the parasitic phase, they are characterized by a morphologically reduced appearance[1,122]. Plaut[171] placed infected skin pieces and infected hairs in moist chambers and observed the formation of morphological structures never seen in the parasitic phase, in scrapings or hairs. Davidson and Gregory[73] repeated these experiments in 1934. They placed detached hairs, naturally infected by *Microsporum audouini, M. felineum,* and *Trichophyton gypseum,* in a van Tieghem cell without any nutrient medium and observed all characteristic morphologic structures (micro- and macroconidia, and others) developing around the hairs in a humid atmosphere.

If the dermatophytes are induced to grow on detached hairs in a humid atmosphere they do not grow in the parasitic phase characterized by a reduced morphology. In that case, the invasion of the hair is performed in quite a different way. Furthermore, the

dermatophytes growing on hairs *"in vitro"* often develop many of the morphological features known from their growth on Sabouraud media such as micro- and macroconidia. Fluorescence, as developed in hairs infected by certain dermatophytes, cannot be observed[214]. These important observations of Plaut[171], Davidson and Gregory[73], and Vanbreuseghem[214] indicate the marked difference in the growth forms of the dermatophytes on living, non-detached keratin (parasitic phase) and on detached, dead keratin (saprophytic phase). Gordon's[103] finding of macroconidia of *Microsporum gypseum* in soil confirms the experiments of Plaut, Davidson and Gregory, and Vanbreuseghem.

Among the dermatophytes, there is a group which parasitizes on man only. These human species are very rarely found in animals[27,54,62,149,184,200]. Their transmission to laboratory animals from cultures is usually hard to perform[62,67,187].

A second group of dermatophytes affects man and animals likewise, the animals usually being a source of infection for man.

Little is known about a third group affecting animals only, because no thorough study of this group has ever been made. The number of dermatophytes on domesticated and wild animals is probably considerable[184].

Why some dermatophytes attack only human keratin and other ones human and animal keratins as well, is not fully understood yet. The differences in the chemical composition of various keratins[29] which the dermatophytes have to digest with their enzymes[71,138,156,207] to establish their parasitic growth phase may well account for this selective parasitism.

The clinical features of dermatomycoses caused by human and animal species are well described and discussed by Sabouraud[187], Miescher[142], Arzt and Fuhs[5], and Kumer[120]. Therefore, we will mention only a few of the marked clinical and epidemiological differences. The human *Microsporum* species, *M. audouini,* is highly infectious and it causes large school and home epidemics. The infection is usually restricted to the scalp. *M. audouini* is of low pathogenicity; kerion formation does not occur very often[130,182], and therefore spontaneous cures are exceptional. However, Rivalier[182] recently reported cases in which spontaneous cure occurred. The most common animal *Microsporum* species, *M. canis,* is of lower infectivity and the reported epidemics have never reached the size of those caused by *M. audouini.* Scalp and glabrous skin are affected likewise. *M. canis* has a higher degree of pathogenicity; kerion formation and spontaneous cures are not uncommon.

Marked differences can also be observed in infections caused by those *Trichophyton* species of human and animal origin which affect hair and glabrous skin as well. Endothrix infections (fungal elements inside the hair only) are due to human species such as *Trichophyton tonsurans, T. Sabouraudi,* and *T. violaceum.* Endoectothrix infections (fungal elements inside and outside the hair) point to the animal origin of the dermatophyte involved. In endothrix infections, simultaneous infections of the glabrous skin are much rarer than in endoectothrix infections. The human species are usually found in children only[170], but the species of animal origin may be found more often in adults. The report by Sá Penella and Roda[193] who observed only 14 cases of kerion formation among 3,309 cases of ringworm of the scalp largely caused by human species is quite representative. As in cases of animal microsporosis, kerion

formation is more frequently induced by animal species of the genus *Trichophyton*. The very large number of cases of endothrix infections (countries around the Mediterranean Sea, Eastern Europe, Asia) may reflect the higher infectivity of the human species. The infectivity of the animal species is decidedly lower; the number of cases in a certain area is always much smaller.

The importance of the dermatophytes of animal origin is clearly demonstrated by the flora of dermatophytes isolated in rural regions of those countries where the human types are not endemic. In such regions, the cases of dermatomycosis contracted from animals are overwhelming, especially if the cases caused by the more contagious *Microsporum* species are not taken into account. Although the clinical material coming to the big centers where the mycological investigations are usually done may be a selected one, the respective reports are quite impressive[4,6,7,21,44,66,68,70,83,90,95,100,105,115,117,130,135,160,167,173,177,197,204,205,206]. The urban flora of dermatophytes, characterized by a large majority of isolated human species, the number of animals and thus the sources of infection being smaller than in rural districts, is well reflected in Sabouraud's report on the dermatophytes which he isolated between 1907 and 1909 at the Hôpital St. Louis in Paris[187]. Forty years later the statistics published by Degos and Rivalier[75] revealed a similar pattern. In another region of France, Gaté, Coudert and Chmel[94,95] found in a series of cases of ringworm, nearly all among shepherds and stockbreeders, 19 cases caused by *T. mentagrophytes* and 2 by *T. album*. Cattle were implicated as the sources of infection in the majority of cases. On this continent, the dermatophytes isolated by Lewis and Hopper[131] from non-epidemic tinea capitis in New York City were almost entirely of human origin.

Two reports from Berlin, Germany[4,116] indicate the prevalence of dermatophytes of animal origin. The most striking feature is the great number of isolations of *T. epilans*, which was very rare among the isolates in Paris. Since the two reports cover the years of the First World War and 1919–1920, it must be assumed that the high percentage of animal species isolated is probably due to the large epidemics of sycosis barbae to which we will refer later. Keller[117] and Pfister[167] investigated the flora of dermatophytes of Oberbaden, Germany, in 1923 to 1926 and 1948 to 1950 respectively. Although the percentage of the isolated species varied during the two periods the pathogenic fungi isolated were almost solely of animal origin in this rural district. Engelhardt[83], Grütz[105], and Schmidt[197], working in different rural areas of Germany, also found that most cases of dermatomycosis were due to contact with animals. The same holds true in rural regions of Hungary[6], Italy[68] and Finland[160]. In Uruguay, Talice and Mackinnon[205,206] did not isolate *Microsporum audouini*. Their cases of microsporosis were entirely due to animal species. Most of the *Trichophyton* species isolated were also of animal origin. The authors interpret the high number of species of the *Trichophyton faviforme* group as the result of the density of the bovine population in their country.

We will now discuss the more important dermatophytes as to their occurrence in man and animals. The older literature, so well covered by Sabouraud[187], Plaut[172], and Bruhns and Alexander[42], will be referred to only occasionally.

MICROSPORUM CANIS. (*M. felineum, M. lanosum, Sabouraudites canis*). *M. canis* and the disease caused by it are

so well known and so often described that we can limit ourselves to a few remarks. The species is more widely distributed than the human species, *M. audouini*, which is very rare in South America[135,151,205]. It may be mentioned that according to Pätiälä[160] microsporosis does not exist in Finland, and Catanei[52] reports that microsporosis is rare in the countries around the Mediterranean Sea. The disease in dogs and its transmission to man has been well studied and described by Sabouraud, Suis and Suffran[190] and by Nicolas and Lacomme[155]. The ambiguous epithet "canis" is misleading since the fungus also causes disease in other animals, such as cats and horses[54,85,110,124,139,154,180]. In a large epidemic in St. Gallen, Switzerland, Zollikofer and Wenner[223] found cats as the sources of infection in 45 cases. A similar report by Saunders[195] describes one kitten as the source of infection for 8 persons, and Sabouraud[189] reports an epidemic caused by one Persian cat and finally involving 25 children and adults because a veterinarian did not recognize the source of infection Sabouraud had suspected. In the fall of 1954, we studied an epidemic of more than 100 cases in a district of Montreal. Cats were found to be more often the sources of infection than dogs[23]. Yet, it must be borne in mind that in many cases the disease is transmitted from man to man[23,142,187], although it is said that the human spread usually stops after 4 to 6 transmissions. Animals may become infected by contact with man. Davidson and Gregory[72] reported the infection of a cat by a child with microsporosis, and we found that a dog contracted a *M. canis* infection from a boy who used to sleep with his infected head on the hip of the shepherd dog[23].

MICROSPORUM EQUINUM. Bodin[30,31] gave the first description of this dermatophyte. It has often been isolated from cases of equine microsporosis[36,38,54,127,196,207]. Lebasque[127] reported that 60% of all cases of ringworm among horses in France were due to *M. equinum*. Bergner[19] isolated the fungus in an epidemic among horses of the German army, and Schermer[196] found it in horses of the German army after the Sudeten campaign and among those coming home from France during the Second World War. Krantz[119] was able to inoculate man with strains he had isolated from horses. According to Sabouraud[184], *M. equinum* only rarely affects man. However, Bang[7] saw 22 human infections during the years 1909 to 1913. Talice and Mackinnon[205] reported one case from Uruguay. In an epidemic outbreak of ringworm among 321 horses of a remount depot, Neefs and Gillain[150] isolated *M. equinum* in 80% of all cases, and also found arms and forearms of attendants infected by the same dermatophyte. Baudet[13] isolated *M. equinum* in severe outbreaks of ringworm in horses in Holland during the Second World War. He found it readily transmissible to man.

MICROSPORUM FULVUM. *M. fulvum* was first isolated from a case of microsporosis in Argentina by Uriburu, who sent the culture to Sabouraud for identification[187]. Unfortunately, the species is usually regarded as a synonym of *M. gypseum*, whereas it should be regarded as a distinct and valid species. Dowding[79] described distinctive differences between the macroconidia of *M. fulvum* and *M. gypseum*. Recently, Rivalier[183] re-examined the original strain of Uriburu kept in the Sabouraud collection of the Hôpital St. Louis in Paris. The strain had not lost any of its morphological characters. Since nobody has ever found a real case of characteristic microsporosis (hair with a heavy sheath of small arthrospores) caused by *M. gypseum*,

Rivalier[183], too, wants to keep *M. fulvum* as a valid species.

In 1926, Ballagi[6] isolated this dermatophyte from human beings and a cat; his identification was confirmed by Sabouraud. Hasegawa and Yamamoto[109] found it in three different monkeys (*Macacus cynomolgus*) and were able to transmit the fungus to man. Pfister[168] recently reported a mixed infection of a foot by *T. interdigitale* and *M. fulvum*. His identification was confirmed by the Centraalbureau voor Schimmelcultures (Prof. J. Westerdijk) in Baarn, Holland.

MICROSPORUM GYPSEUM (*Achorion gypseum, Sabourandites gypseus*). Sabouraud[187] probably first isolated this dermatophyte from a boy and his dog in 1894. At that time he placed the organism in the genus *Trichophyton*, although he had already noted the similarities with *M. fulvum*. Bodin[32] gave the first detailed description, and since he had isolated the fungus from a case with scutula formation (favus), he identified it as *Achorion gypseum*, because the other organism causing favus (*T. Schönleini*) was placed at that time in the now obsolete genus *Achorion*. Langeron and Milochevitch[123], basing the classification on morphological features, recognized the dermatophyte as a *Microsporum* species (*Sabouraudites gypseus*).

Lebasque[127] described the infected equine hair as endo-ectothrix. The fungus, able to produce a variety of lesions, does not seem to have caused a case of microsporosis[183].

M. gypseum has been isolated from horses[19,36,38,54,59,127,150,196,210], monkeys[111], dogs[187], cats[3,176], a tiger[202], and a chicken[3]. Ajello[3] to whom we owe a very complete review of the literature, found only 270 cases of human infections reported up to 1953, a surprisingly small number since the dermatophyte has been isolated on all continents. Rabut and Dumet[177] described lesions on the arm of a patient, and Trice and Shafer[212] reported an infection of a patient and his dog. We have isolated this dermatophyte during the years 1948 to 1950 only twice, in Switzerland[21], from tinea corporis, but during the years 1951 to 1954 five times from human beings and once from a horse in Canada[23]. One of these human cases was a mixed infection of the glabrous skin, caused by *M. canis* and *M. gypseum*.

TRICHOPHYTON EPILANS (*T. cerebriforme, T. flavum, T. plicatile*). *T. epilans* has often been confused with or regarded as synonymous with *T. tonsurans* (*T. crateriforme*). Sabouraud[187], who did the first isolation, already stressed the difference as far as the infected hair is involved: *T. epilans* causes an endo-ectothrix-, *T. tonsurans* an endothrix infection. Bodin[31], Sabouraud[187], and Vuillemin[219] suspected the animal origin of the species, and circumstantial evidence (higher frequence among patients from rural areas and frequent isolation from sycosis barbae[4,6,41,68,105,115,116,130,151,173]) supported the suspicion. Sabouraud[187] believed that an unidentified fungus isolated from a child and a cat of the same house might have been *T. epilans*[43]. Later Plaut[172] isolated it from cats, and Negroni[153] from horses in the Buenos Aires region.

The dermatophyte was very frequently encountered in Germany, especially in the northern part, during the time before, during, and shortly after the First World War[4,105,107,108,115,116,117,167,173]. In 1916, Lewandowsky[130] reported from Hamburg that 75% of all cases of dermatomycosis in the north west of Germany were due to *T. epilans*. *T. epilans* was largely responsible for the epidemic outbreaks of sycosis barbae in Germany. It is

interesting to note that Götz[104] did not find the dermatophyte in the Hamburg area during the years 1948 to 1950 among 168 cases. Bang[7] found *T. epilans* in 112 of 311 cases of ringworm in Denmark. A similar percentage was reported by Dalla Favera[68] from Italy. From Hungary, Ballagi[6] and von Berde[15] reported a relatively high percentage of *T. epilans* infections in rural districts.

TRICHOPHYTON MENTAGROPHYTES (*Ctenomyces mentagrophytes*). Many authors place all the dermatophytes described by Sabouraud as *T. asteroides, T. granulosum, T. radians,* and others, in one single species, *T. mentagrophytes.* In this review, we will refer to these dermatophytes as they were named in the original papers.

De Lamater[77] reported an infection of the squirrel. Ringworm of muskrats, caused by *T. mentagrophytes,* was described by Errington[84] and Charles[63]. Errington and his assistant became infected while investigating the disease affecting up to 25% of the herd. Catanei[54] isolated *T. mentagrophytes* from dogs and horses, Pinoy[169] and Emmons[82] from monkeys. The fungus was found in mice, which transmitted the infection to both hands of a laboratory technician[34]. We made a similar observation and isolated the dermatophyte from the palms of three laboratory workers handling white rats in a research laboratory[23]. Ceresole[60] described a ringworm epidemic among fallow-deer in Italy. The fungus was also transmitted to the warden and 12 persons of the park where the fallow-deer were being kept. The human lesions were found on legs, hands, cheeks, and neck. Rabbits were the sources of infection for 14 persons in Spain[218].

According to Walker[220] *T. mentagrophytes* is common in Great Britain. Suspected sources for her human cases were laboratory mice, rabbits, cows, sheep and rabbit skin. Mink skin was proved to be a source of infection. The unusually high incidence of *T. mentagrophytes,* not *T. interdigitale,* causing tinea pedis and tinea corporis in British troops serving in Malaya and Hong Kong[192] deserves special attention. It is reminiscent in many respects of the reports by Pautrier and Rietman[163,164]. It is interesting to note that neither Vanbreuseghem[215] nor Clarke and Walker[64] mention any species of this group in their surveys of the flora of the Belgian Congo and Nigeria.

T. ASTEROIDES (*Ctenomyces asteroides*). Sabouraud[187], who isolated the organism from a number of human cases and a horse, reported an infection of a French officer. He had slept on blankets used by horses showing ringworm. Brocq-Rousseu[36] found *T. asteroides* in horses, and Muende and Webb[147], in horse dung. An explosive outbreak of ringworm in mice of the Wellcome Physiological Research Laboratories was described by Parrish and Craddock[159], where in addition to hundreds of mice, two horses and three laboratory attendants were affected. Infections in silver foxes and their attendants were reported from Norway[69,91]. During the First World War the number of *T. asteroides* infections in Berlin, Germany, decreased with the departure of the horses from the city to the battlefields[88]. Catanei[53] found dogs infected by the fungus in Algeria. In Denmark, Sylvest[204] recorded an increasing number of human cases parallel to the increasing number of cattle infections by this dermatophyte. Forty years earlier, Bang[7] had already described many human cases in that country. Other human infections were reported from Hungary[6,15] and Great Britain[81,209]. *T. asteroides* was often found in cases of dermatomycosis in rural areas of Ger-

many[117,167,197], and Bruhns[41] frequently isolated it from sycosis barbae.

TRICHOPHYTON RADIOLATUM (*Ctenomyces radiolatus*). In addition to the cases described by Sabouraud[187] those reported by Engelhardt[83] should be mentioned. The latter author isolated *T. radiolatum* from a cow and found it in three generations of the owner's family, in which the grandfather had transmitted the infection to his daughter and granddaughter.

TRICHOPHYTON GRANULOSUM. (*Ctenomyces granulosus*). *T. granulosum* was first isolated and described by Sabouraud[166] in an epidemic affecting 800 horses of a French cavalry regiment in Sedan. The horses showed lesions on all parts of their bodies. During this large epidemic, only one ringworm-like lesion was observed on the finger of a soldier. Since only two human infections by the fungus[68] had been recorded up to the publication of Sabouraud's classical book "Les Teignes"[187], Sabouraud came to the conclusion that *T. granulosum* might cause epizootics in animals, only occasionally becoming parasitic for man. This surprising fact seemed to be of great importance from the epidemiological point of view, since the *Trichophyton* species of animal origin, though more virulent to man than those of human origin, did not become so adapted, even when they appeared to have an opportunity as in the case of the epidemic in Sedan.

Fischer[89], in 1917, described 17 human cases in a German hospital. Clinical evidence suggested that the small epidemic affecting nurses and patients was probably started by two soldiers who had returned from the battlefields in Northern France. Two years later, Gutmann[107] reported a similar series of human infections in another hospital.

That *T. granulosum* is able to become acclimatized to man was reported by Pautrier and Rietman[162] in 1922. These authors described the first large epidemics in human beings in two mental institutions in France (Stephansfeld, Bas-Rhin and Rouffach, Haut-Rhin). One hundred inmates were affected, showing skin lesions which very often became generalized. In the same year, Pautrier and Rietman[163] reported on isolated cases, among which was one in which a mother, daughter and their dog were infected. A third paper by the authors[164] described an infection of a laboratory worker following washing of tubes containing very old and dried cultures. In a fourth paper[165], Pautrier and Rietman recorded a case in which, besides lesions of the smooth skin, nine finger nails were involved.

Brocq-Rousseu, Urbain and Barotte[38] isolated *T. granulosum* from horses. It was also isolated repeatedly from chinchillas, showing "fur-slipping," and from chinchilla breeders[23,24]. Du Bois[80] observed an epidemic in mice. The fungus was found in cattle, sheep, and farmers in Finland[160,161], and in human beings in rural districts of different countries such as Hungary[6], Germany[167,173], Denmark[7], Switzerland[21], United States of America[102], and Canada[23].

TRICHOPHYTON LACTICOLOR (*Ctenomyces lacticolor*). Two cases of human infection by this dermatophyte with kerion formation were described by Sabouraud[187]. Ballagi[6] isolated *T. lacticolor* from an infection of the beard. Guinea pigs were found to be infected spontaneously[152], and Brandt[35] described how 22 persons became infected from a horse.

TRICHOPHYTON PERSICOLOR (*Ctenomyces persicolor*). *T. persicolor*, with its peach-colored colonies, is one of the rarer dermatophytes. Sabouraud[187] found it in 3 cases, but believed that it might have been seen first by Adamson. The fungus was usually iso-

lated from hand and wrist and other exposed parts of the glabrous skin[81,115, 117,143,145,191,204,213,220]. The most interesting fact was the high incidence (10 of 51 isolations) Keller[117] observed in Oberbaden, a rural district of Germany. All the isolations originated from isolated cases. Pätiälä[160] found *T. persicolor* in a dog in Finland, and Vanbreuseghem[213] was able to inoculate animals with the dermatophyte. Walker[220] isolated the fungus four times during the years 1946 to 1949 in Great Britain and in one of her cases, a horse was suspected to be the source of infection. Recently, we have isolated *T. persicolor* from thigh and legs of a child who used to ride on the back of a horse with ringworm-like lesions. Unfortunately, we were not able to get infected material from the horse or from two other children who had ridden on it and showed the same type of lesions as the first child[23].

TRICHOPHYTON RADIANS (*Ctenomyces radians*). Sabouraud[187] described an infection of a veterinarian who specialized in the treatment of small animals. Cats were implicated in a large epidemic raging in government offices in Paris between 1923 and 1926[179]. Two hundred cases of ringworm due to *T. radians* were found among women working in these offices. Careful investigations lead to the conclusion that mice infesting the offices infected cats which chased them. The cats transmitted the disease to the office workers, but there does not seem to be any doubt that the dermatomycosis spread from person to person too.

TRICHOPHYTON DENTICULATUM (*Ctenomyces denticulatus*). Plaut[173] described an epidemic among packers of a plant in Hamburg. However, the report does not mention the origin of the packing material, suspected of being the source of infection, neither does it state how the material might have become infected. Further cases were reported from Southern Hungary[15].

TRICHOPHYTON MEGNINI (*T. rosaceum*). *T. Megnini* is common in north western Europe: Holland[148,157, 158]. Belgium[208], Northern Germany[4,90, 104,107,108,115,197], Denmark[178,204], and Finland[160]. According to Muijs[148] and Papegaaij[157,158], 90% of all cases of sycosis barbae seen in Amsterdam, Holland, were due to this dermatophyte. Fischer[90] found it in 24% of his cases in Berlin, Germany, and claimed that the fungus invaded Germany from Holland and Belgium[89]. Rasch[178] reported 22 cases of sycosis barbae as caused by *T. Megnini*. He suspected that the dermatophyte had been imported to Denmark from Germany. Twenty-two years later, *T. Megnini* was found only once by Sylvest[204] in Denmark. That a similar disappearance of this dermatophyte from the British flora occurred, must be concluded, if the surveys by Duncan[81] and Walker[220] are compared with earlier reports[42]. Dalla Favera[68] isolated the fungus in Italy.

Muijs[148] described a family epidemic. The father had an infection of the beard, the son ringworm of the scalp and two daughters had lesions on the glabrous skin, a clinical picture usually seen in family epidemics due to contact with cattle[25]. Therefore, Schmidt's findings[198] were not surprising at all. He examined cows on farms where cases of sycosis barbae had occurred; 7 out of 18 cows investigated were found to be infected by *T. Megnini*, whereby the long suspected animal origin of this dermatophyte became established.

TRICHOPHYTON EQUINUM. *T. equinum* was often isolated from horses[13,23,36,38, 110,127,134,150,196,202,211]. According to Lebasque[127] the number of infections caused by *M. equinum* is greater (60%) than those by *T. equinum* (28%). Pätiälä[160] found *T. equinum* in a herd

of cattle in Finland, which provides another argument against using an epithet with exclusive reference to the first discovered host.

Man may become infected by *T. equinum*. In an epidemic described by Tornabuoni[211] from Italy, 200 horses were found to be infected, and 20 persons contracted ringworm from the horses. Mackinnon[134] also observed direct transmission from horses to man as did Neefs and Gillain[150]. Other human cases were reported by Kaufmann-Wolf[116], Beare and Cheeseman[14], Walker[220], Finn[86], and Mackinnon[135]. An epidemic in people handling packing material was seen by Plaut[173].

TRICHOPHYTON FAVIFORME GROUP (*T. verrucosum, T. album, T. discoides, T. ochraceum, T. immergens, T. langeroni*). The animal origin of the species of this group was recognized long ago[185]. The study of their nutritional requirements by Langeron and Milochevitch[123], and Georg[96,97], not only revealed a more complete morphology besides "formes de souffrance"[123], they also lead to better methods of isolation[99]. Ainsworth and Georg[2] proposed the use of Bodin's old epithet "verrucosum" anew. Bodin's[31] thought was that this group formed a linkage between other *Trichophyton* species and the causative organism of human favus, *T. Schönleini*. Sabouraud[187] disagreed, but Langeron's findings[121] that hairs from favus patients may also show the typical endo-ectothrix-mégaspore form of parasitism as caused by the faviforme group, lend weight to Bodin's hypothesis.

Sabouraud[185] rightly called these dermatophytes "parasites des bovidés." In France, ringworm of cattle is due in 53% of all investigated cases to *T. album; T. discoides* causes 35% and *T. ochraceum* 12% of infections of cattle[127,128]. Besides bovines[9,10,14,22,25,54,68,106,110,118,125,144,198,202,204,220] other animals might be sources of infection: horses[10,23,30,36,38], goats[10,58], dogs[11], sheep[54], donkeys[30], a dromedary[8], and a canary[136] have all been mentioned in the literature. Bodin[30] described how a horse imported from Denmark to France infected all 40 other horses in a stable. The epidemic lasted 3 months. Nine persons became affected during its course.

Lesions in human beings are usually found on the hands and forearms, but may occur on any other part of the glabrous skin. Kerion formation is not uncommon. Beard infections in farmers are frequent. Ringworm of the scalp is found in children and adults. We have also seen infections of the eyebrows and eyelids.

Transmission to man was often reported, especially in farmers and their families, cattle-dealers and persons in related professions[6,14,20,22,25,30,45,68,83,92,93,100,114,125,167,204,220]. Guimaraes and Lacerda[106] were able to transmit *T. album* experimentally to man by slight scarification, the lesions developing after 21 days. Transmission from man to man was also observed[25].

The geographical distribution of the *Trichophyton faviforme* species seems to be worldwide. Georgjevic and Milochevitch[100] reported that 33.6% of all ringworm cases in Yugoslavia are caused by such species. Ballagi[6] and von Berde[15] found many cases in rural districts of Hungary. The same holds true for Italy[68] because of the density of the bovine population. In Paris[75,187], such cases are rare. Sylvest[204] found an increasing number of infections due to these species, which coincided with an increase of animal cases. *T. discoides* is quite common in Great Britain[220] and Northern Ireland[14]. Many reports from North and South America indicate the large number of human infections due to this

group of fungi[20,25,45,74,92,93,106,131,151,206]. Boedijn[33] found a species of this group in Java, as did Mancy[137] in Australia. Neither such experienced workers as Catanei[52] nor Vanbreuseghem[215] mentioned these species in their surveys of the flora of dermatophytes of Algeria and the Belgian Congo. In other instances, the absence of these dermatophytes from reports on ringworm diseases might sometimes be due to the difficulties encountered in the isolation of these *Trichophyton* species.

TRICHOPHYTON QUINCKEANUM (*Achorion quinckeanum, Sabouraudites quinckeanus*). The long known *T. quinckeanum* is usually regarded as the causative organism of favus in mice. Vanbreuseghem[216] was able to transmit this mouse favus from culture to man and even found formation of scutula on the person he had inoculated. Judging from the numerous reports in the literature, human beings easily contract ringworm caused by *T. quinckeanum*[6,7,57,61,76,81,86,87,116,126,158,173,174,197,199,201,220,222].

The dermatophyte may even cause epidemics. De Jong and van Nederveen[76] reported that Dutch soldiers became infected while staying in a school infested with mice. Chajes[61] found more than 20 girls infected by *T. quinckeanum;* they all worked in a fur store where many scabby mice were seen. In Australia[65], men working in wheat stacks contracted ringworm. *T. quinckeanum* was isolated from their lesions and from mice trapped in the wheat stacks. Plaut[173] and Lawrence[126] gave examples of direct transmission of *T. quinckeanum* from mice to man. Schneider[199] investigated an epidemic in a German laboratory where field mice transmitted the fungus to a dog. Then, several persons contracted the disease from the dog. Wittels[222] found 3 cases in one family, apparently contracted from a cat, and Soltmann[201] in one case suspected a horse as the source of infection.

TRICHOPHYTON GALLINAE (*Achorion gallinae, Microsporum gallinae, Sabouraudites gallinae*). *T. gallinae* was first described by Mégnin[140,141], but is often confused with *T. Megnini*[98,186]. It causes favus in poultry[56,175,181]. Pätiälä and Härö[161] isolated the fungus from a black cock. Baudet[12] found *T. gallinae* on a dog apparently infected in a chicken house. The number of human cases reported is very small. Sabouraud[187], who was able to induce lesions in human beings, reported one case. In 1926, A. Sartory, Petges, and R. Sartory[194] described a generalized infection of the glabrous skin due to *T. gallinae.*

All the dermatophytes so far mentioned have been shown to be of animal origin. They infect man and animals alike and may be transmitted from one to the other. The human species, usually, are isolated from man only. However, a few exceptions in the literature may be referred to.

Sabouraud[184] once found *Microsporum audouini* on a dog, but raised the question whether the dog was not infected by a boy. A simultaneous infection of a boy and a dog was also observed by Murrell[149]. Later, Scully and Kligman[200] reported that several people contracted a *M. audouini* infection from a 5-month-old pet capuchin monkey. It is very difficult to transfer *M. audouini* to laboratory animals. Sabouraud[187] only once obtained a positive result when he planted an infected hair of a child in a hole he had made with a needle in the skin of a guinea pig. Courmont[67], on the contrary, claimed successful inoculation of guinea pigs, rabbit and horse; Truffi reported similar success in a letter to Sabouraud[187].

In 1911, Bloch[27], isolated a dermatophyte from 4 persons who, among

other lesions, had developed scutula (favus). Since the fungus formed a violet pigment, Bloch named the fungus *Achorion violaceum*. The infection of these 4 persons had been contracted from mice. Sabouraud[188] did not find any marked differences between the cultures of *A. violaceum* Bloch had sent him and those of *T. violaceum*. The latter was successfully transmitted to monkeys, dogs, cats, guinea pigs, cows and horses[42,46,48], but is regarded as a human (endothrix) species.

In rare cases, the causative organism of human favus, *T. Schönleini*, may be found on or contracted from animals such as mice[184], rat[113], hedgehog[184], cat[202], and dog[53,54]. Sabouraud[187] believed that the number of infections contracted by man from animals infected by *T. Schönleini* is rather negligible.

Recently, dermatophytes, isolated by Chakraborty and Ghosh from two cows and a dog, were identified by us as *T. rubrum*[62]. To our knowledge, it was the first time that this fungus, usually causing infections of the smooth skin and nails, has been reported as isolated from animals. It is not surprising that the isolation was done in India, since *T. rubrum* originates from the East[122] where it is very common. Ghosh, Panja and Dey[101] found it in 63% of their cases of dermatomycosis. Could not the increasing number of reports of sycosis barbae and tinea capitis due to *T. rubrum*[26], [132,133,135,137,146,220,221] (the beard infections are usually due to animal species) point in the direction of animal origin? So far, the difficulties encountered in the experimental transmission of *T. rubrum*[62] from cultures to animals do not substantiate this possibility.

A long time ago, Sabouraud[187] was able to grow *Trichophyton* species on rotten wood, ears of wheat, barley and oats, on grains and soil. Similar experiments were done by Brocq-Rousseu, Urbain and Barotte[39,40]. In one experiment, they were able to show that a culture of *T. mentagrophytes* of equine origin on straw was still alive after 27 months and that a completely desiccated culture of *M. equinum* on a poor medium remained viable after 3 years. Both cultures had kept their virulence and were still able to produce typical lesions in animals. Catanei[47] working with *T. radiolatum* confirmed these reports. The long lasting vitality of *M. gypseum*, *T. asteroides* and *T. quinckeanum* was also observed by Hruszek and Hruszek[112], that of *T. granulosum* by Pautrier and Rietman[164]. Muende and Webb[147] described how *T. asteroides* was isolated from horse dung in a stable populated previously by cattle. The horses did not become infected, but 14 calves which took the places of the horses all became infected. We observed that in one stable the cows attached to a certain wooden pillar always became infected by *T. discoides* while the rest of the herd remained healthy[23].

The isolation of virulent *M. gypseum* from the soil by Gordon[103] and Ajello[3] deserves special attention. Gordon collected it from soil which probably had been exposed to chicken droppings. Ajello, using hair as bait[217], was able to get more isolates on sites either obviously populated by animals or on areas frequented by domestic and wild animals.

Using membrane filters, Gordon found macroconidia of *M. gypseum* in the soil. It shows that the fungus develops a more complete morphology in soil and outside living keratin than in the reduced parasitic phase on man and animals. Furthermore, it points to the saprophytic origin of the dermatophytes, already postulated by Bodin[31] and Sabouraud[187].

These facts indicate that animal dermatophytes may persist and keep their virulence outside the body, on such material as soil, straw, wood, especially if mixed with shed-off keratinaceous material. It might well explain unexpected explosive outbreaks of dermatomycosis in animals[24,159,166] and man[162,192].

Animal infections occur more often than is commonly supposed. They can cause economic damage: hides fall in value[16,17,18], milk production drops and loss of weight can be observed in cattle affected by severe lesions[22]. Pelt-bearing animals, such as silver foxes[69,91] and chinchillas[24], readily diminish in market value due to fur-slipping if infected by dermatophytes. The number of human infections of animal origin is quite considerable, as has been shown here, therefore it is of utmost importance to search for and recognize the sources of animal ringworm to prevent further spreading, and even the threat of legal procedures[189]. The recognition of the animal origin of ringworm in man is also of value for the determining of an appropriate treatment.

Thibierge[208], trying to draw conclusions from all dermatological experiences during the First World War, found, when he tabulated all the reports of severe sycosis barbae among the Central Powers, that dermatophytes of animal origin were the cause of these epidemics, a fact completely overlooked at the time of the epidemics.

Many aspects of animal ringworm are not yet understood. It is mainly due to the fact that the dermatologists and medical mycologists are usually working in the big centers, often far away from the sources of infection. The selected clinical material found in these big centers can easily lead the clinician to underrate the animals as a reservoir of dermatophytes. A hundred years ago, Letenneur[129] already deplored the fact that dermatologists were limiting their activities to large centers only. An even greater danger is the "lumping" of species by mycologists in order to reduce the number of species. Such "lumping" "may conceal interesting epidemiological and clinical data."[1] Therefore, any progress in this field will depend upon exact mycological diagnosis; but it also requires a close cooperation of physician, veterinarian and medical mycologist.

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PREVENTIVE MEDICINE AND EPIDEMIOLOGY

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CERVICAL CANCER AS A MASS DISEASE

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To speak these days of an epidemiology of cancer occasions no particular surprise, nor does it arouse undue curiosity. The doctrine of disease as a mass phenomenon is as well established as the concept of the clinical event. Whether concerned with prevention or treatment, medical practice of course has more to do with that unit of observation which is a single person, than with situations where a population serves in that capacity. For one thing, there are more individuals than populations; and secondly, the current of medical thought has been directed for years toward the patient rather than the community.

So far as the development of new knowledge is concerned, experimental pathology has outweighed other methods of approach to such extent that the remaining two procedures, clinical investigation and epidemiologic analysis, are often slighted and sometimes ignored.

As epidemiology eventually escaped its conventional restriction to epidemics and infections, the study of the group and of disease as a mass phenomenon came to include a variety of morbid conditions having an impact on human populations. There was good reason for this. Some disease processes are completely important as they affect the individual, rabies for example, but of relatively little moment in respect to total populations. Conversely, a disease of minor concern to the affected person may be of major importance to a population, the common cold for example.

With no intent to suggest the epidemiologic method as anything more than an adjunct to the other two broad means for study of disease, morbid processes do exist of such obstinacy in

yielding the facts necessary for treatment and control that complete justification is had for bringing up all available artillery in their engagement. Cancer is such. The main effort in cancer research is through laboratory study, and rightly so; and yet the bulk of such measures as exist for prevention and control are of clinical and epidemiological origin. Cancer was one of the first fields to engage the attention of epidemiologists as effort turned to the broader fields of mass trauma and the mass phenomena of degenerative, neoplastic and nutritional diseases, intoxications and others, in what has been termed a modern perspective of epidemiology[34d].

Progress toward an understanding of cancer as a mass disease was for many years fitful and unorganized, and more statistical and demographic than epidemiologic. Only in the past decade has there been concerted effort to incorporate epidemiologic studies in the programs of institutes for cancer research, to attract field investigators trained in epidemiologic method, and to devote adequate resources to this part of cancer research. Cancer investigators, other than those with a primary concern of field method, have expressed the opinion[100b] that studies on etiologic factors are underemphasized, compared to those on the already established tumor. The history of mass disease shows that control has more often come through measures specifically directed against established causative factors than through a full understanding of the biologic nature of the process. What has thus far been gained in control of cancer, and it has been none too great[72], has been mainly through the clinical methods of diagnosis, prognosis and treatment. The issue here is the potentiality of the epidemiologic method to supplement facts gained from clinic and laboratory, toward an improved understanding of cause and a better prevention; by observing the natural history of cancer as evidenced in populations of people, and by making use of those counterparts of clinical methodology which in epidemiology are prevalence, course, and control. Each of the three basic methods of study has selective usefulness. For example, prognosis in respect to the clinical case has only relative exactness; with a population as the unit of observation, a reliable rule is established.

CANCER EPIDEMIOLOGY. The mass relationships of the group of diseases collectively known as cancer have had attention for many years, even antedating the time that malignancies came to be recognized soundly as a public health problem[9]. The start of a public health concern in the United States may be dated with some assurance from 1898, when the State of New York[80] appropriated the sum of ten thousand dollars to discover the cause and cure of cancer. In developing this interest it was reasonable to make use of the epidemiologic techniques[33c] which had served so long as the diagnostic discipline of public health[34e] where effort was mainly toward control of infectious diseases.

Using records of deaths from cancer in Massachusetts, which are to this day among the more reliable sources of information, Whitney[119], in 1901, was concerned with the question of an increasing frequency of cancer. The data from 1850 to 1900 showed progressively more deaths from this cause but the increase was not wholly regular; periods of favorably low rates occasionally alternated with an excess incidence. The long-term upward trend was attributed in part to better diagnostic procedures and better reporting of deaths, but some part was seemingly inherent in the disease itself and was considered

to represent an actually augmented frequency.

Reliability in diagnosis of cancer has always been a problem, particularly for inaccessible as contrasted with accessible sites of origin. A study by the Bureau of the Census[111], 1914, gave the highest certainty of diagnosis to cancer of the rectum, while cancer of such inaccessible sites as ovary and fallopian tube was much less, and for cancer of the stomach decidedly low. Willcox[120] of Cornell enlarged an earlier study by British workers[56] of cancer in Frankfurt-am-Main for the period 1860–1889, by bringing observations to 1915 and including pertinent data of American origin. Both studies were concerned with increasing rates as influenced by improved diagnosis and a change in age composition of the population. The conclusion in common was of no probable actual increase. With this opinion Schereschewsky[95] disagreed, on the basis of the behavior of cancer mortality in the ten original registration states of the United States for the period 1900 to 1915. A portion of the increase could not be explained by any or all of the stated factors. Time settled the matter, for an increasing frequency of cancer as a cause of death is now clearly evident. An effective control requires demonstration of the responsible factors.

An epidemiology of cancer received measurable stimulus by reason of an outstanding Massachusetts "Special report of the Departments of Public Health and Public Welfare, relative to the prevalence of the disease of cancer throughout the Commonwealth and particularly of the disease in its inoperable stage or form" by Bigelow and Conant[6], Boston, 1926. A stated objective was "learning more regarding the disease from an epidemiologic standpoint" which soon materialized through the thorough-going field studies of Bigelow and Lombard[7b]. Charles V. Chapin[13], so regularly in the forefront of epidemiologic advances, was the first in this country to consider cancer mortality by economic status. Centers for epidemiologic study of cancer eventually came with establishment of a Cancer Section[71] of the Massachusetts Department of Public Health, in 1926; of a division of Cancer Control[78] in the New York State Department of Health in 1931; a branch of Biometrics and Epidemiology in the National Cancer Institute[112] and increasing emphasis on this activity by the Bureau of the Census[69] and now the National Office of Vital Statistics, Public Health Service, Department of Health, Education and Welfare. Cancer registries to gather records of morbidity came into being in Massachusetts[7a] in 1927; in Connecticut[16] in 1935, and in the State of New York[79] in 1940.

The epidemiologic study of cancer is nevertheless a neglected field[100b] in comparison with the effort expended on clinical and laboratory investigations, but enough information has accumulated to permit Levin[59b] and Gilliam[33b] to bring together the principles of the epidemiologic method as applied to cancer; and Levin has reviewed the epidemiology of cancer from the standpoint of the health officer[59a]. An extensive summary of illness from cancer in the United States has been assembled by Dorn[25b], and of mortality rates by Gover[36].

In Great Britain, progress in interpreting cancer as a mass disease closely paralleled developments in America. The early work of King and Newsholme[56] has been mentioned. In the 1917 Annual Report of the Registrar General of England and Wales, Stevenson[102] gave thorough consideration to whether or not cancer was increasing in the British population. Mortality for cancer of accessible sites among

males had increased faster than that for inaccessible sites, and the reverse was true for females. The general increase among women was measurably less than for males. The greater rates for males thus appeared less a matter of better diagnosis and more an actual increase of the disease. Greenwood's review[39a], in 1928, of recent statistical studies of cancer problems was followed shortly by a chapter in his textbook[39b] on the epidemiology of cancer, this being the first time the subject attained that dignity. The recent account by Stocks[103b] of cancer deaths at different ages in England and Wales, 1921 to 1950, has general interest.

CANCER IN RELATION TO OTHER MASS DISEASES OF MAN. The relative importance of cancer among mass diseases of man is readily established by its rank of second among the ten leading causes of death in the United States. Essentially one of every seven persons dies of cancer. Reported deaths[114a] for 1950 were 210,733; and estimates for 1953 are in excess of 225,000.

Cutler and Haenszel[22] likewise estimate the number of cases for 1953 to be of the nature of 530,000 and for 1975 the prospect is about 753,000, by all standards a conservative value. They advance as a more satisfactory measure of the cancer problem an estimate of the probability of developing cancer by a specified age or during the future lifetime of a person. When observed age-specific cancer incidence rates[77a] are applied to the 1950 life table, roughly 50 million people alive in 1950 can expect to develop cancer within their remaining life, and one-third of new-born children will sometime have cancer.

TIME-PLACE RELATIONSHIPS. Cancer mortality in terms of reported deaths per 100,000 population has been steadily increasing for many years, this being true with proper age-standardized rates to take into account the altered proportions of older persons in the population. The data for the State of Massachusetts (Fig. 1), have a series that dates from 1850. Information for the United States as a whole, available for the years 1900 to 1951, are in essential agreement.

Mortality for cancer of all forms, and for all ages past 30 years, has increased in the United States and in Great Britain since 1850. The enlargement still continues, with the most rapid increase in the period 1860 to 1915, and a tendency in recent years for rates to level off, especially for females.

Much of the recorded increase in recent years is man-made, in that a greater proportion of existing cases are recognized through improved diagnosis and by reason of case-finding programs. An aging population has much import. More extensive exposure to recognized carcinogens is likely responsible for the increase in some forms of cancer. A changing genetic pattern of susceptibility has yet to be demonstrated.

With due regard for differences in recognition and reporting, cancer varies in observed frequency from place to place. This group of diseases broadly considered, is more frequent in northern United States than in the south; the rates tend to be higher in the east than in the west. The differences between countries are still greater, a characteristic recognized since the early summary of mortality from cancer throughout the world by Frederick L. Hoffman[47] and brought to date by the World Health Organization[122c] for 1951.

The groupings of countries and the changes with time are such as to suggest that cancer is a sickness which increases with the spread of material civilization. The disease is clearly a more frequent cause of death in this country than it was a generation ago.

Mortality rates for western countries completely overshadow those of more primitive states. The reasons, however, are more deep seated than the state of civilization; they transcend matters of cultural advantage or economic ascendancy. The things that follow in the wake of improved living are the critical consideration. A people with a life span of 32 years scarcely reaches the cancer age. A low social state is frequently identified with a high cancer rate, age being constant, but the variables associated with that state are the answer, not the state itself.

SEX DIFFERENTIAL. The established pattern of more deaths from cancer among women than men held for so many years that it came to be considered immutable. A likely although far-distant change in this relationship was evident almost a hundred years ago, for the mortality among men increased at a relatively constant rate which with scattered exceptions was at a greater pace than for women (Fig. 1). The two trend lines came closer together and eventually crossed in 1941 in Massachusetts. The behavior since then would seem to fix the new relationship as permanent. Cancer mortality thus joins the general death rate in being greater for men than for women; it has followed the same long course as did tuberculosis, where female preponderance was once definite

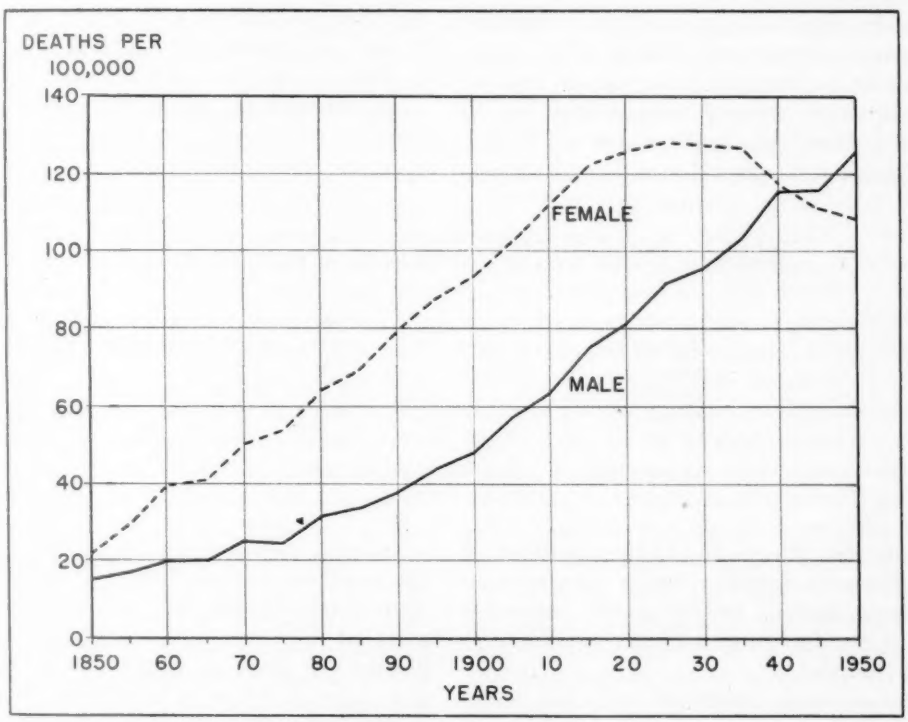

Fig. 1.—Cancer deaths per 100,000 population, by sex, Massachusetts 1850–1950; average annual rates for 5-year periods, age-adjusted to population of Massachusetts 1900.
Source: Division of Cancer and Chronic Diseases, Massachusetts Department of Health[70].

but now has changed to a characteristically male disease.

The result in cancer occurred despite the markedly greater risk of women developing cancer of the genital organs than men. Among all cancers of women, malignancy of the genital organs (Table 1), has a higher probability by age 65 years than does carcinoma of the breast and is exceeded only by malignancies of the intestinal tract as a life-time risk. An adequate explanation of why cancer of the genital organs occurs so frequently in women and infrequently among men would aid in understanding the basic causes of malignant new growths. A knowledge of why the change in relative position of male and female deaths occurred in Great Britain twenty years before it did in the United States would also be helpful.

TABLE 1.—PER CENT WHITE WOMEN EXPECTED TO DEVELOP GENITAL CANCER BY AGE 65 AND DURING FUTURE LIFETIME

Beginning at	*By Age 65*	*During Future Lifetime*
Birth	4.5	7.2
Age 20	4.7	7.4
Age 45	3.8	6.6
Age 65	...	3.4

Source: Cutler, S. J., and Haenszel, M. A.[22]

CANCER BY SPECIFIC SITE. For some purposes and in general definition of the problem, all cancers usefully may be considered together, but in the search for causative factors, and in analysis of course and behavior as a mass disease, that way of proceeding is no more productive than an epidemiologic consideration of grouped diseases having the common characteristic of a rash. Too much then depends on the relative proportion of measles and typhoid fever, or whether or not typhus is included.

As judged by numbers of deaths, which is the most practicable index, the conspicuous forms of cancer differ in epidemiologic characteristics to such extent that each becomes a separate problem. The variation is essentially as great as among the common communicable diseases. Incidence and fatality differ materially for cancer of the skin as contrasted to cancer of the lung. Variations with place are common. Host populations behave differently, as illustrated by the different timing in change of sex preponderance for fatal cancer in Britain and the United States.

An attempt to separate cancers according to specific etiologic agent is currently unrealistic. Cancers caused by a specific carcinogen are known, but the number is small, they are far from representative and they fail to include most of the common malignant neoplasms of man. A second possibility is to base decision on histological manifestations or characteristics. Despite increasing practice of biopsy and greater numbers of necropsies, a pathological classification scarcely fits with a study of cancer in its community relationships. A clinical classification by site of origin, or organ primarily affected, is most practicable as knowledge now stands.

All primary cancers of a given tissue are often of a single kind according to existing criteria; a dominant proportion of cancers of an organ commonly conform to rule. In other circumstances,

kinds of cancer affecting the several parts of an organ are sufficiently different to require further separation.

EPIDEMIOLOGIC ANALYSIS. The first need in attempting to define the mass behavior of a morbid process is to settle the limits of the disease entity with as much exactness as knowledge permits. The requisite of a single disease rather than a group of conditions has been emphasized. Omission of essential parts of a disease process has led in the past to serious error in interpreting course and behavior of the mass disease. Membranous croup, faucial sore throat, chronic nasal excoriation and cutaneous ulcer may be viewed independently but all enter into clinical diphtheria. Inclusion of other diseases, for example, typhoid fever with typhus, confused ideas of cause and transmission for many years. With site the criterion for separation, the first problem in cancer of the uterus is whether to consider malignancy of the body and the cervix as one disease or two, and furthermore to establish the place of cancer-in-situ.

Objective. Epidemiologic analysis has a straightforward objective: to determine how and why diseases have come to be as they are, to behave as they do and to occur in the places they are found. No more is involved than the scientific method applied to a particular purpose, the definition of health and disease as expressed in groups of people. The precise aim in this instance is to recognize those persons or groups of persons, those fractions of a total population, most likely to develop cancer of the cervix and the circumstances that bring that about. Quantitative measurement serves then to define the extent of risk for groups possessing certain characteristics. The way to prevention is then open.

Conceptual Scheme. With epidemiology interpreted as medical ecology[34a], wherein mass disease is but one of the many results of a total interaction between man and his environment, the resolution of a mass disease into its component elements becomes an ecologic analysis[34c], with separation of contributing factors into three primary categories, those having to do with an agent of disease, with the host attacked and with the environment in which both are found. Facts derived from field study, and from clinic and laboratory, are set down in ordered fashion and their relationships examined as they contribute to cause and course of the mass phenomenon. The cause of mass disease is seen as multifactorial. Two broad causal systems are distinguished[35]. The first consists of a series of interrelated variables of which one, commonly an agent of disease, is a necessary requirement. Subdivisions include that system which depends upon the presence of an agent, as in lead poisoning or syphilis; secondly, that marked by the absence or deficiency of the agent as in ariboflavinosis; and finally, that where an agent conceivably exists but is unknown. The second general system is that of an association of events or factors in such combination and at such time that disease results, no single constituent being invariably essential and active. In both instances the combination of factors responsible for genesis of the process is open to wide variation and expectedly deviates from one situation to another. Cancer of the uterus is viewed as having its origin within the second type of causal system. Epidemiologic analysis aims to define occurrence according to time, place and person, to measure variations in course in terms of frequency and severity, and to permit development of hypotheses warranting test for validity in a search for cause.

Methodology. The manner and means by which facts are collected in the field to test hypotheses of causation

or to confirm types of behavior is outside the scope of this review. The subject has had recent thoughtful treatment by Gilliam[33b] in respect to cancer. Investigation follows two general patterns. The proband method is one. The start is with a case of the disease and a satisfactorily matched control. Series of cases and controls are assembled. Differences and similarities between the two groups are sought in explanation of causes and course of the disease. The second general procedure begins with a total population, giving consideration to those who do and do not have the disease. The study may be prospective or retrospective. The two population fractions are examined as before for differences in host and environmental characteristics that bear on the origin and behavior of the disease. In general, the proband method is of advantage in studies of a disease of low incidence. That it will ever settle basic problems of the epidemiology and genetic determination of cancer is improbable. Such studies do lead to useful hypotheses and occasionally are productive of methodological advance. Studies of total populations, cohort studies with longitudinal histories or long-term study of families[27] have the greater promise. They are costly and require years of observation. The emergence of causative relationships, however, is anticipated as slower in leisurely developing organic disease such as cancer, than in the epidemics that arise from infectious agents. Furthermore, a century is a long time for an individual person but short for a population; and populations are the concern of epidemiology. These long-term observations seem to be the main promise in settling a goodly number of the critical group problems associated with cancer, primarily those of risk, its nature and cause. The approach is epidemiological.

Old books have a charm, and the information they give of disease in other eras and other places is not to be discounted in proper understanding of what those diseases are today. Greenwood[39b] speaks revealingly of "dusty official reports which have thrilled me." A statistical approach marked the beginnings of an epidemiology of noninfective disease[25c]. Statistical analysis of records of deaths often serves well in determining the magnitude of a problem, but the other items these records contain are so few and of such irregular value that a contribution to knowledge of causative factors is necessarily restricted.

That a health problem exists is the usual indication of a need for new knowledge. When a community is concerned, that knowledge is to be sought best on the highroads and in the homes of people; and when the investigator steps out into the field, that is no longer a statistical maneuver. Numerical techniques are essential to evaluation of most collected results, clinical, laboratory and epidemiologic, but they are neither one nor the other discipline. There is an art to epidemiology as in clinical medicine; if this were not so, epidemiology would be biostatistics and clinical medicine a mere matter of biochemistry.

Basic Epidemiologic Data. Deaths are one source of material, cases another. The main dependence in cancer is on deaths because of greater reliability of the data and more general availability. Death records have inherent shortcomings. Adequacy of clinical diagnosis has improved, biopsy has become more general and necropsies more frequent, but differences are so great in different time periods and different places that comparison is unsatisfactory. Standards of practice from one country to another are still more divergent. Other causes of death ac-

tually may be responsible for deaths attributed to cancer, although, in general, numbers are likely to be understated.

Deaths are an inexact measure of case incidence for survivors are not counted[93] and even for cancer they are a factor, in some forms more than others. Data on deaths before 1900 are generally unreliable. Classifications change and statement of cause is subject to altered custom. All cancers of the genital organs were long lumped together and only, in 1938, was a serious attempt made to separate cancer of the uterus from others of the female genital tract. The sixth revision of the Manual of the International Classification of Diseases, Injury and Causes of Death[122a], 1948, recommended specific report of this cancer as of cervix, corpus and others, but practice in the United States is still such that the results are far from the reality suggested by clinical experience.

Morbidity data on a country-wide basis are more or less limited to Denmark; for the world, information does not exist. That provided in the United States for a few areas and some sampled populations has appreciable limitations. Useful information is accumulating from the three well-established state cancer registries in this country and something from a number of others, but with the same faults as for all reporting systems and that includes notification of the communicable diseases. The registry data on cancer are scarcely comparable between states because methods of collection vary. Massachusetts, for instance, collects full case histories from state-aided cancer clinics and hospitals, with an annual check on all known cancer patients and deaths of that source. Connecticut receives reports from almost all general hospitals of the state. New York requires report of cancer cases by physicians, hospitals, and laboratories, exclusive of New York City, in much the same manner as for communicable disease.

Field surveys are the preferred method for assembling morbidity data but with the weaknesses inherent to effort in chronic disease. The information provided by the person interviewed is regularly less extensive and less exact than for acute processes which by their nature attract the greater attention. The beginnings of the condition are likely to be forgotten. Because of its nature, cancer often induces prejudice and disinclination to give information. The canvass of diagnostic facilities and a correlation with reported deaths as carried out in the Ten-City Study[26] is a good procedure but thus far has had limited application and to selected populations. Other points of departure in location of cases are through community roentgenographic surveys for lung cancer; or in cancer of the cervix, through vaginal cytologic examinations[87].

The discussion now turns to the reasons for considering cancer of the cervix as an independent mass disease. Epidemiologic manifestations of the entity are then presented, along with various associations relating to selected features of host and environment.

Cancer of the Female Genitalia. The greater part of the excess cancer of the female genitalia, compared with that of sexual organs of the male, rests in the high susceptibility of the uterus. Malignancies of two sites are concerned, cervix and endometrium, the latter here designated body of the uterus but including anatomical subdivisions of fundus and corpus, all else than cervix. Other primary genital cancers of women are those of ovary, fallopian tube, vulva and vagina. Cancer of the cervix is easily the most common, as judged by reported deaths. Involvement of the

corpus uteri is second and the others have minor importance. The relative rank of genital cancers and the position among other cancers of women is shown for 4,444 cancer deaths of women in Massachusetts during 1950 (Fig. 2).

CANCER OF THE CORPUS UTERI. The features that distinguish cervical cancer from that of the corpus are clinical, pathological and epidemiological.

Clinical. Physicians have long regarded cancer of cervix and corpus as separate clinical entities[29]. An etiologic agent of cancer of the corpus uteri is unknown. Factors suggestively contributing to causation have been listed[19] as endocrine dysfunction, associated disease processes, parity and age of the patient, and constitutional features which include obesity, hypertensive state and sterility. A social environmental influence is seen in the excess frequency of cancer of the endometrium reported by gynecologists[37] among well-to-do patients seen in private office practice.

Patients with endometrial carcinoma cease menstruating later than normal women[21,117]. Metrorrhagia[11] tends to precede the late menopause, with the hypothesis advanced[52] that continuous endogenous and exogenous stimulation is a consistent feature in causation.

Recognition of cancer of the body of the uterus is more difficult and less exact than for cancer of the cervix. Its characteristics are essentially those of

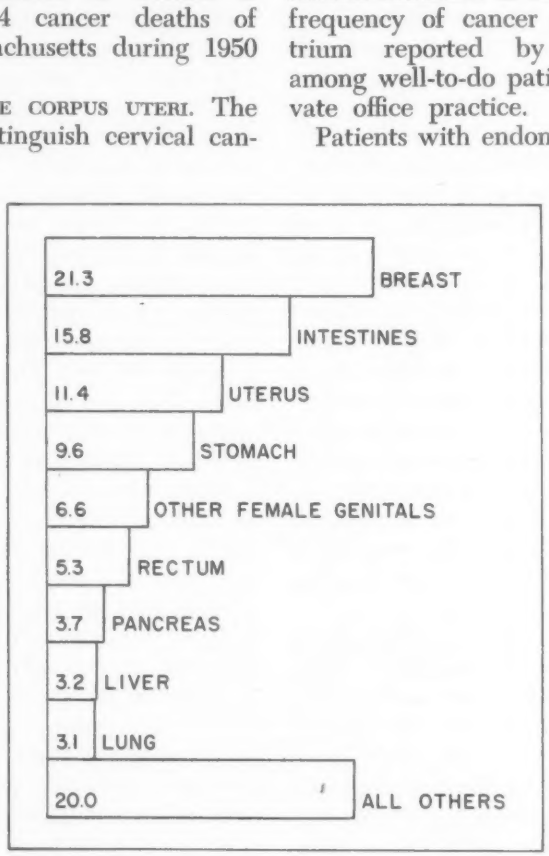

Fig. 2.—Per cent distribution of 4,444, cancer deaths among women, Massachusetts 1950, by site.

Source: Division of Cancer and Chronic Diseases, Massachusetts Department of Public Health[70].

other internal cancers. The development of a cytologic test by Papanicolaou[87], based on abnormal patterns of cells, variability in size and shape and of staining characteristics in material obtained by cervical-vaginal smears have proved useful, but less so than for cancer of the cervix.

The difficulties in diagnosis are reflected in the range of opinion about relative frequency of the two conditions. Rates per 100,000 females from recent case incidence studies show a 2.3:1 ratio of cervix to corpus for white populations (Table 2), and about twice that value for non-white. Ratios arising from clinic and hospital series range from 2.4:1[67] to as much as 9:1[85], with recent reports[18] tending toward the closer ratios but not invariably so[74]. Any presumed increase in cancer of the endometrium is explained in part by the aging population of women and by improved diagnostic measures.

Pathological. Cancer of the body of the uterus is not a pathologic entity. The predominant type is adenocarcinoma, but mesothelial malignancies of connective tissue and squamous cell cancers are encountered[43] as well as malignant neoplasms originating in the stroma[30]. Cancer of the cervix, by contrast, is mainly a squamous cell carcinoma[109].

Epidemiological. A later age of attack for cancer of the body of the uterus than for cervix is an observation of long standing and abundantly established[19,117]. The pathology of the lesion seems to be a determining factor in the average age at death which for adenocarcinoma, whether of cervix or fundus[40], is some five years later than for squamous cell carcinoma of the cervix where the ordinary range is from 45 to 50 years. Age of attack in cancer of the corpus conforms to that of cancer in general, especially that of inaccessible sites, leaving the implication that exogenous factors of the environment have more of a part in causation of cervical than of endometrial cancer. The fatality of cervical cancer is appreciably less[73b] than for cancer of the corpus, accessibility and greater ease in diagnosis being one determining factor, with some suggestion that biological differences in tumor behavior also act[83b]. Mortality rates are measurably greater for cervical cancer because of the greater incidence.

TABLE 2.—CANCER OF CERVIX UTERI AND CORPUS UTERI, CASES PER 100,000 FEMALES PER ANNUM, IN TEN SELECTED CITIES OF THE UNITED STATES, 1947, AND IN THE STATE OF IOWA, 1950; AGE ADJUSTED TO 1950 U. S. FEMALE POPULATION

	Cervix	*Corpus*	*Ratio Cervix/Corpus*
TEN CITIES:			
White	39.1	16.8	2.3
Non-white	78.8	16.2	4.7
All races	43.7	16.6	2.6
STATE OF IOWA:			
All races	36.6	19.3	1.9

Source: Haenszel, W., and Marcus, S. C.;[41] Dorn, H. F., and Cutler, S. J.[26]

The evidence from all three sources justifies distinction of the two conditions of cervical and endometrial cancer as independent mass disease processes.

Cancer of the Cervix Uteri. Again with the obligation to define the clin-

ical limits of the disease cancer of the cervix before examining mass relationships, the immediate problem is to dispose of cancer-in-situ. The question is whether or not to include it within the biologic gradient[34b] of what is accepted to be the disease entity, cancer of the cervix.

CANCER-IN-SITU. This condition, also known as intraepithelial carcinoma, has been defined[83b] as "a lesion in which the entire thickness of the squamous epithelial layer is replaced by cells microscopically indistinguishable from those of frank invasive cancer, with complete loss of stratification but with no invasive penetration of the basement membrane." It is an asymptomatic condition, recognized only by microscopic examination of involved tissue. Although occurring in other organs, the principal concern is with lesion of the cervix and its possible place as a preinvasive stage of frank squamous cell carcinoma. A variety of evidence supports the contention that it is.

At least 54 instances[57] have been reported where invasive cancer followed previous recognition of carcinoma-in-situ by biopsy. Carcinoma-in-situ often has been identified in the edges of invasive cervical cancer[50]. It occurs at an earlier patient age than invasive carcinoma (Fig. 3) with 30 to 34 years the mode at time of recognition, an observation corroborated by Younge and Hertig[124], in contrast to ages 50 to 54 years for invasive cancer.

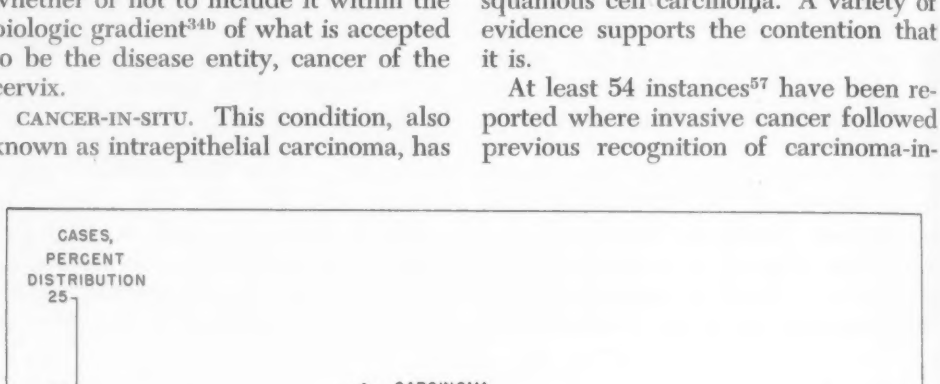

Fig. 3—Per cent age distribution, 242 cases of cervical carcinoma-in-situ (intraepithelial), and 208 cases invasive cervical cancer from 912 biopsies of 60,000 women examined. Memphis, Tennessee Cytology Project.
Source: National Cancer Institute[77b].

Invasive carcinoma of the cervix

and carcinoma-in-situ have a number of epidemiological characteristics in common. Comparing 100 cases of cancer-in-situ with 225 of invasive squamous cell carcinoma[91] the frequency was in both instances greater among Negroes than whites, although the average age at detection was somewhat less for Negro (39.5 years) than for white patients (43.2 years). None of the 100 cases of carcinoma-in-situ occurred among Jews. Of 381 Jewish women admitted in 5 years to the gynecological service of Western Reserve University Hospitals, only one had proven carcinoma-in-situ; the frequency for 5,181 women of other religious preference was 0.93%.

A general population of Tennessee, primarily urban but including the surrounding district, when surveyed by vaginal cytological techniques gave a prevalence of cancer-in-situ and of invasive cervical cancer, by age groups for the first 60,000 women examined, as illustrated in Fig. 3. The age concentration for carcinoma-in-situ was earlier than for invasive carcinoma. The plan as described by Dunn[28] is to repeat examinations of the same population at yearly intervals, thereby determining incidence of invasive cancer and of carcinoma-in-situ. The obvious implication is that if cancer-in-situ is proved to be an early phase of invasive cancer, that is the stage of the process at which recognition is important since treatment of the lesion would offer a method of mass prevention of frank cervical cancer. Whatever the result, information will be provided on the incidence of these two neoplasms and on the nature of cancer of the cervix in its early stages.

Evidence discounting this relationship comes from observations[48,94] that lesions microscopically similar to carcinoma-in-situ are to be found in nonmalignant conditions such as pregnancy, healing of erosions and even in degenerative and inflammatory processes such as the postmenopausal state and in trichomoniasis. A part of the difficulty may be in lack of uniform diagnostic criteria among pathologists.

An answer to the question of whether carcinoma-in-situ usually progresses to an invasive lesion is difficult, since once the diagnosis is made, surgical treatment is usual. Younge, Hertig and Armstrong[124] state, moreover, that the entire lesion of carcinoma-in-situ is sometimes removed in one biopsy and, therefore, the diagnostic procedure then amounts to treatment of the disease. However, Kottmeier[57] claims that only 8 of 53 untreated cases of carcinoma-in-situ followed for 10 years became invasive.

The evidence on progression of intraepithelial to invasive cancer is suggestive, but as yet too inconclusive for the condition carcinoma-in-situ to be viewed as other than a separate process. In further discussion of cancer of the cervix, attention is confined to invasive carcinoma and the intraepithelial lesion is excluded.

Invasive Cancer of the Cervix. The frequency and gravity of cancer of the cervix led Novak[83a] to characterize the disease as probably the most important of all gynecological conditions. The disease may develop before or after menopause, and unless treated early is almost certain to end fatally. The first signs usually are bleeding or abnormal vaginal discharge. Since involvement of parametrial lymphatics tends to occur early, the disease often has extended beyond the cervix by the time symptoms present. Characteristically, bleeding when it occurs is intermenstrual, traces of blood being noted after coitus, douching or straining at stool. A watery discharge frequently precedes bleeding, especially in adenocarcinoma, but sooner or later becomes

blood-tinged. Bleeding is due to destruction or ulceration of tissue and consequently becomes more frequent and profuse as the disease progresses, leading to marked anemia if blood loss is severe. Pain is not usual until late stages when nearby bladder or rectum are involved, either by direct extension or by lymphatic spread. Severe pressure upon the ureters, or actual invasion of these structures, sometimes results in hydroureter and hydronephrosis; fistulæ of bladder or rectum are other complications. As in other cancer, loss of weight and general debility characterize advanced stages.

Necropsies have demonstrated that cancer of the cervix may spread to almost any tissue or organ of the body[73a]. Death is usual, however, before distant metastasis, by uremia from blocking of the ureters, hemorrhage from eroded blood vessels, or septicemia from secondary infection.

FREQUENCY IN THE UNITED STATES. The incidence of cancer of the cervix in the United States is unknown. In recent years the Public Health Service has made two extensive field surveys to obtain a basis for reasonable estimates. The first survey, in 1937, and again in 1947, concerned an urban population of about 14 millions from ten selected metropolitan areas deemed representative of the United States. The cities were Atlanta, Birmingham, Dallas, New Orleans, San Francisco, Denver, Philadelphia, Chicago and Detroit. Cases were collected from hospital records, from physicians and through records of death.

The second surveyed population was that of the State of Iowa, a typically rural region, and for the year 1950. The results are given in Table 2. The rates are in close agreement with those for white populations of the urban areas, a result rather to be expected since the population of Iowa is predominantly white. If further experience proves these rates representative, the incidence of cancer of the cervix for the United States as a whole is somewhat in excess of 40 per 100,000 female population per annum.

Deaths are an unreliable index of incidence of cases except for those few diseases of man such as rabies and acute leukemia where attack regularly ends in death. They have more value in cancer than for most others, but among cancers less for cervical carcinoma than for many because the recovery rate is relatively high, although much less than for cancer of the skin.

The difficulty is further intensified in that only since 1949 has concerted effort been made to report death from cancer of the cervix as such. Until that time report was commonly of cancer of the uterus and data are for all malignancies of that organ; deaths from cervical cancer can only be estimated. Specific report of cancer of the cervix is, of course, wholly desirable but thus far has proved of no practical value. Total reported deaths from that cause were 8,336 in 1950, a rate of 11 per 100,000 female population. The rubric "other and unspecified" included 7,749 deaths, a proportion wholly outside any reasonable clinical or epidemiological experience. Reported deaths do have value in indicating trends: short term trends with considerable reliability and long term trends where the quality of recognition and reporting is consistent.

Reported deaths in the United States from cancer of the uterus, all forms, increased from 13,959 in 1930, to 16,085 in 1950. Total deaths obviously may increase because of more population or more aged people, with rates per unit of population actually less. It is therefore not unexpected that dating from 1947[114a] and for the four succeeding years, mortality rates for cancer of

the uterus have progressively declined.

Crude death rates[114b] from this cause were fairly constant over the past 20 years: 23.3 in 1930, 24.3 in 1935, 24.3 in 1940, 23.6 in 1945, and 21.2 in 1950, a moderate downward trend being discernible since 1940. Corrected for age, on the basis of the female population of 1950, the differences are still greater and indeed since 1930 rates show a continued downward succession for each 5-year period through 1950, the extremes being 29.9 in 1930, and 21.2 in 1950.

Since cancer of the cervix is the dominant cancer of the uterus there is little doubt that death rates for cancer of the cervix have also declined. Cancer of the cervix is furthermore an accessible cancer which with modern methods of treatment results in recovery of as many as 60 to 70% of patients[18,73a] provided the condition is recognized while still confined to the cervix. Two inferences may be drawn from the apparent decrease in rates of death from cancer of the cervix.

Effective treatment has more than offset the greater number of cases coming to notice by reason of improved diagnostic facilities or arising from the risk inherent in an aging population.

It also seems reasonable that if cancer of the cervix is related to a definite carcinogen, the agent or agents are not of recent origin or use; otherwise the death rate would be expected to show a steady increase over the past 20 years, as has cancer of the lung in males[114b].

Attention now turns to an ecologic analysis of cervical cancer. Features and characteristics of the human host and of the natural environment will be searched for evidence or suggestion of interaction influencing frequency and behavior. Host resistance and susceptibility are considered in terms of physiologic function and anatomic structure, of growth and development, and of inherent genetic constitution; environment in respect to its physical, biologic and social components. Interaction is conceivably either between some one component of host and another of environment, or more broadly within the matrix of the total causal system to involve multiple and various combinations of host and environmental factors. The objective is identifiable factors in causation susceptible to preventive action. The question of a true agent of disease, acting in production of cervical cancer, is first examined.

AGENT OF DISEASE UNKNOWN. Demonstrable agents of disease are variously physical, chemical and biologic. No specific thing, substance or object has been demonstrated to act regularly in causation of cancer of the cervix, either by its presence or its absence. On the basis of what is known, the causal system responsible for the disease is that of the second order earlier stated, wherein interaction is between factors of host and environment and lacking the *sine qua non* of a particular element recognized as the direct and inciting agent. However, the question of a specific agent or agents can not be dismissed with complete assurance. Three features of the general biology of malignant growths warrant consideration; they bring no proof but do suggest that the concept of an agent is compatible with some observed features of cancer of the cervix.

The age at which patients develop cancer from exposure to known carcinogenic agents depends on the age at which exposure begins, the intensity and duration of exposure, the potency of the carcinogen and on individual susceptibility[49]. Cancers due to demonstrated carcinogens tend to occur at an earlier than average age, with scrotal cancer of children employ-

ed as chimney sweeps the classical example. Cancer of the cervix is also seen to occur at younger ages than does cancer of the corpus uteri or indeed most other cancers (Fig. 5). The data are in keeping with an exposure to exogenous carcinogens for cancer of the cervix, and a lack of such exposure in cancer of the corpus.

Epidermoid carcinoma has been advanced as the prevailing form of cancer where a carcinogenic agent is involved[123]. The greater proportion of epidermoid cancer of the lung is among men; and among women, adenocarcinoma prevails. The association of cancer of the lung in males and an exogenous agent is familiar reading these days. Squamous cell carcinoma is recognized as the predominant cancer of the cervix, and adenocarcinoma of the corpus uteri.

Carcinogens newly introduced into the environment should lead to an upward trend in deaths, which has not been true for cervical cancer. If an agent exists, the problem is not that of looking for new agents but of searching for old ones. A variety of materials has been suggested as containing agents capable of acting on cervical mucosa as carcinogens to produce cancer of the cervix. They include smegma, microbial flora of the vaginal tract, contraceptives and coal tar douches.

Smegma. If smegma has carcinogenic capacity, cancer of the cervix and of the penis should be infrequent among peoples practicing early circumcision of males. Mosaic law prescribes that the male Jew be circumcised on the eighth day of life. Moslems practice circumcision between the sixth and ninth year.

Both cancer of penis and cervix are rare among Jews, with only two reports of penile cancer[23,121] known. Cancers of cervix and penis are common among Hindus who do not practice circumcision. Khanolkar[55] at the Tata Memorial Hospital in India cites 2,511 male Hindu patients with carcinoma, of whom 3.4% had cancer of the penis, compared to 0.2% of 835 Moslem patients. In the same study, 44% of 929 Hindu women with cancer had cervical carcinoma as compared to 20.7% of 275 Moslem cancer patients (Fig. 4). Parsees provide a contradiction, although marriage is later than for Hindus and presumed length of exposure therefore less. Although not practicing circumcision, cancer of the cervix was less frequent than for either Moslems or Hindus: 14.8% of 169 female cancer patients. Data are not given for penile cancer of Parsees.

Wynder *et al.*[123] found an association between low levels of cervical cancer and intercourse with circumcised husbands, using data derived from cervical cancer patients of American and Indian hospitals. For general populations of the two countries, smegma formation by the uncircumcised was greater the lower the social class.

The widely quoted reference to Handley[42b] that smegma may lead to differences in bacterial flora of the vagina in women having intercourse with noncircumcised sexual partners, and hence more cervical cancer, is simple speculation: a note to the editor. Supporting data[42a] are neither given nor are they known. That smegma of man contains a carcinogen is equally unsupported, although whole horse smegma[90] and the nonsaponifiable fraction have led experimentally to skin cancer in mice.

The observed association between circumcision and cancer of the cervix has not been demonstrated under a sufficient variety of conditions and in adequate numbers to warrant acceptance in causal relationship. The hypothesis has the merit and promise to

place it high among field studies awaiting further attention.

Contraceptives and Coal Tar Douches. Contraceptive materials introduced into the vagina conceivably may contain carcinogens or cocarcinogens[5]; or oppositely, condoms and diaphragms could act protectively if there is anything to penile hygiene and smegma.

India. Demonstrated coal tar carcinogens such as methylcholanthrene have produced carcinoma in mice when applied to cervical[76] and vaginal epithelium[31]. Douching materials often contain coal tar derivatives and hence the suggested relation.

Vaginal epithelium is exposed as much or more to action of the suggested carcinogens, or to others unde-

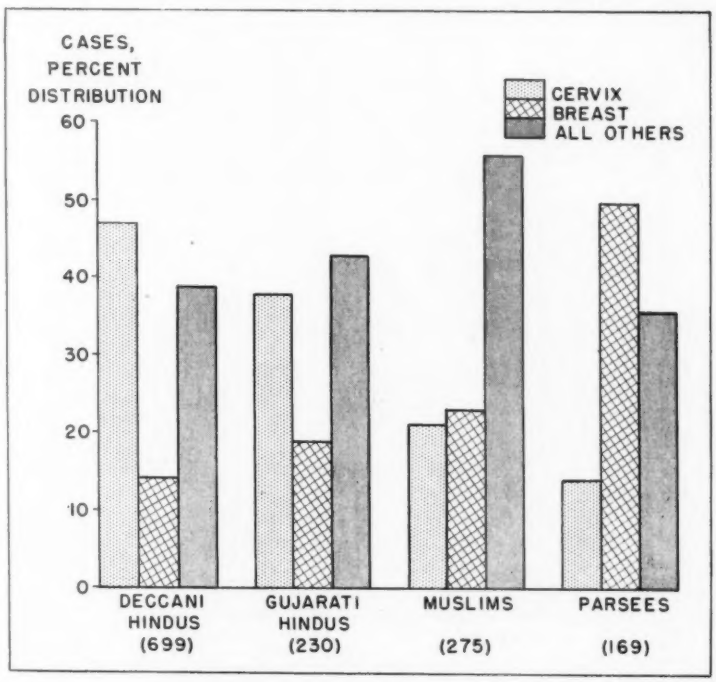

Fig. 4.—Cases of carcinoma, female, admitted to Tata Memorial Hospital, Bombay, India, 1941–1945, cervix, breast and all others, by religious groups.
Source: Khanolkar, V. R.[55].

From study of 549 case histories of cervical cancer, Lombard and Potter[64a,b] concluded that prolonged use of douches containing coal tar products was of questionable significance, with which Wynder *et al.*[123] agree.

Douching and contraceptives are used rarely in many places where cancer of the cervix is common, notably

fined, and yet cancer of the vagina is rare. Either vaginal epithelium possesses a natural resistance lacking in cervical epithelium, or other than extrinsic agents are the determining factor in cancer of the cervix. Although concept of an agent has logical support in a number of features of this disease, proof is lacking. The reasonable course

in search of factors amenable to prevention is to turn toward features of the causal system potentially resident in host and environment.

HOST FACTORS. The capabilities of the human host in contributing to the origin and frequency of cancer of the cervix theoretically rest in such physiologic characteristics as hormonal activity, in processes of growth and development such as those related to age, in an acquired susceptibility or resistance derived from presence of other disease processes, or in an inherited genetic constitution.

Age. Comparative age distributions of cancer of the cervix uteri, corpus uteri and breast from Danish morbidity

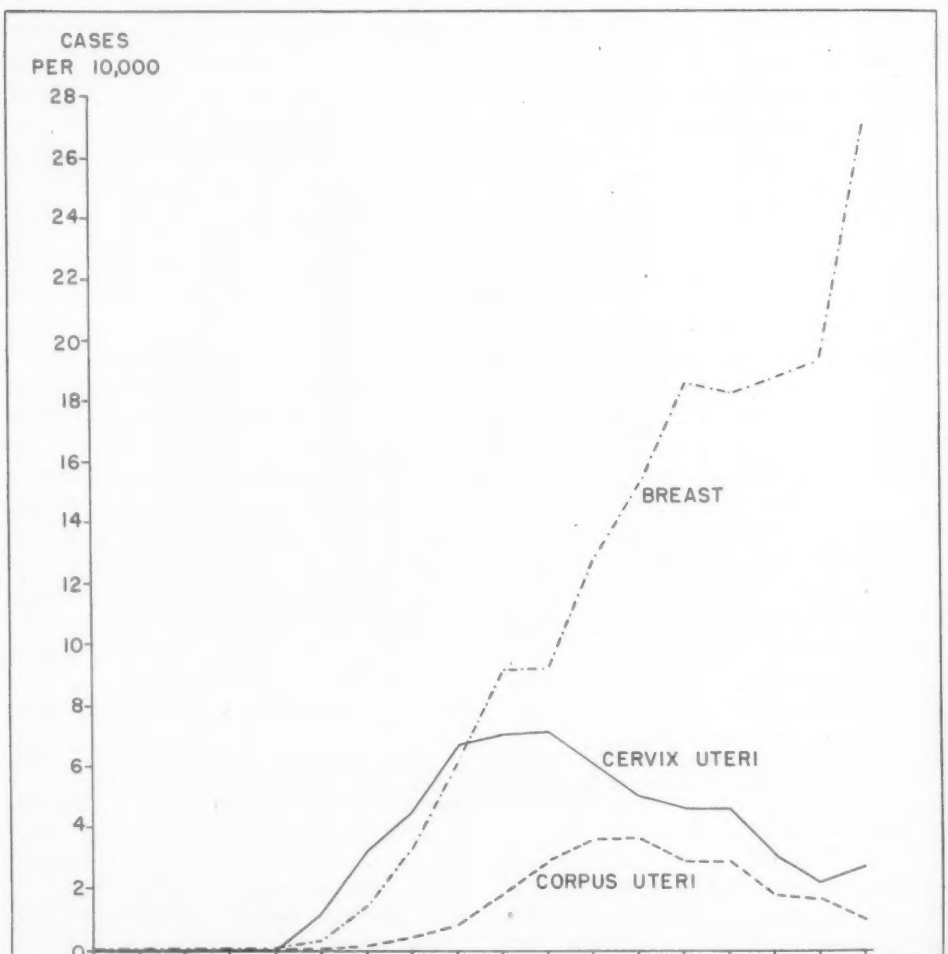

Fig. 5.—Cases of cancer, female, cervix uteri, corpus uteri and breast by age, Denmark, 1943–1947.
Source: Clemmesen, J., and Nielsen, A.,[15b].

experience of 1943-47 (Fig. 5), show a modal age for cervical cancer at about 45 to 50 years, for cancer of the corpus about 10 years later, with incidence of carcinoma of the cervix greater than that of corpus at every age. The frequency of both declines after the modal peak, markedly for cancer of the cervix, less so for corpus. Such decreased incidence with increasing age is unusual among cancers. The upward trend of cancer of the breast does flatten out temporarily at the age when downward trend of cancer of the cervix begins, but rather promptly resumes its upward course. Clemmesen[14b] interprets this behavior as a reflection of change in hormonal status during menopause.

Race. Experience of the United States gives good evidence of racial differences in frequency of cancer of the cervix, the incidence among whites being measurably less than for non-whites. In the Ten-City Study[26] of the Public Health Service combined cases of the 1947 Survey give an incidence of 78.8 per 100,000 females for the non-white population and 39.1 for white. Incidence of cancer of the corpus uteri was the same for both races: 16.2 and 16.8 respectively (Fig. 6). In all, cancer of the uterus among non-white females exceeded that for

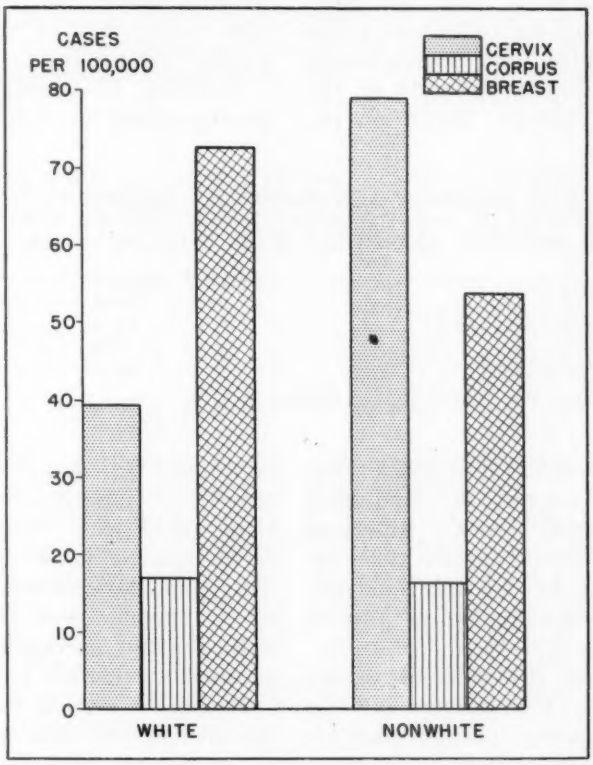

Fig. 6.—Newly diagnosed cases of cancer, cervix uteri, corpus uteri and female breast, white and non-white, Ten-City Study, 1947, age adjusted to U. S. female population, 1950. Source: Dorn, H. F., and Cutler, S. J.[26].

whites by about 60%[25c], while cancer of the breast was more frequent among white females.

Cervical cancer also occurs at an earlier age[26] among non-whites; 40.6% of 703 patients were under age 45, compared with 25.9% of 2,659 white patients. Cancer of the corpus below age 45 was much the same for both.

Reliable morbidity and mortality rates are lacking for other races, but hospital data give some indication of probable differences. Records of the Indian Health Service, based on patients of 48 Canadian hospitals during 1948 to 1952, suggest the frequency of cervical cancer among American Indians to be much higher than expected, judged by survey incidence rates of a general Canadian population for 1948 (Table 3).

TABLE 3.—OBSERVED AND EXPECTED (MORBIDITY SURVEY,[116] SASKATCHEWAN, 1948) CASES OF CANCER: BREAST, CERVIX AND OTHER GENITALS: AMONG CANADIAN INDIANS, 1948–1952

Site	*Observed Cases*	*Expected Cases*	X^2
Breast	26	25.99	0
Cervix	83	8.66	638.15
Other genitals	22	16.20	2.07

Source: Warwick, O. H., and Phillips, A. J.[115]

Similar hospital studies from Malaya compare relative frequency of this cancer with cancers of other sites for Chinese, Indian and Malayan fractions of the population[97]. Among 500 cancers of Chinese women, cervical cancer predominated with 29.4% and breast was second with 17.6%. The 192 cases among Indian women were 29.1% cervical, with buccal cavity and pharynx ranking first among sites with 36.5%. Of 72 Malayan women with cancer, skin was the most frequent site; breast, second; and cervix, third; the proportions being 26.4%, 18.1% and 15.3%.

Differences in frequency distribution among 262 female Bantu patients of Africa[46] were of still another order; cervical cancer accounted for 47.3%, corpus only 1.2% and breast 6.5%. Negro women of West Africa had breast as the most frequent cancer, 21.9%, cancer of uterus 20.3% and liver 17.2%[24].

Female cancer patients from 32 hospitals of Japan for the period 1948 to 1952, included 9,260 or 49.7% with cervical cancer and only 260 or 1.4% with malignancy of the corpus uteri, a rather astounding ratio of 36:1[97].

Heredity. Consideration of a genetic predisposition in explanation of susceptibility to malignant diseases of man[20,45,65a] has turned in many directions; that it is a general biologic factor can be taken as demonstrated. The particular concern here is whether all or specific sites are involved genetically and particularly what is the position in relation to uterine cancer.

Brobeck[10] reports that cancer, irrespective of site, was half again as frequent among relatives of 90 probands having cancer of the corpus uteri as among relatives of controls. Study of 200 probands with cervical cancer led to much doubt that this form was affected by hereditary factors. Sisters of probands had uterine cancer somewhat more frequently than their controls but not cancers of other sites. In Clemmesen's opinion[14a] the increased incidence of uterine cancer among female siblings was better ascribed to exogenous factors than genetic, on the assumption that sexual behavior of sis-

ters was more likely to be similar than that of mothers and daughters.

In a study of 200 probands, cancer of the cervix, Murphy[75a] reported heredity as significant in determining frequency of cancer of the uterus, although quantitatively the differences were borderline. Evaluation of possible direct relation of cervical cancer to endometrial cancer was not possible because of the practical difficulty of differentiating cervical cancer from other malignancies of the uterus among relatives studied.

spring mice to experimentally induced cancer. Law[58] found a lesser incidence of leukemia among mice of a susceptible strain as age of mother at time of parturition was more advanced. Bittner[8] observed the opposite in mammary adenocarcinoma. Chemically induced fibrosarcoma in Strong's experiments[104] appeared with greater or lesser frequency as strains of mice and parental age were varied. Susceptibility to fibrosarcoma was also affected by litter seriation, with the effect considered not to be genetic, although

TABLE 4.—SYPHILIS AMONG WHITE FEMALES WITH REPORTED CANCER OF UTERINE CERVIX AND OTHER SITES, NEW YORK STATE, 1940–1941

Cancer Site	*Number of Cases*	*Syphilis*	
		Number	*Percentage*
Cervix	930	36	3.9
All other	3680	41	1.1
Total	4610	77	1.7

Source: Levin, M. L., Kress, L. C., and Goldstein, H.[60]

In Macklin's[65b] experience with 75 cases of cancer of the cervix no marked genetic effect was determined, cervical cancer being perhaps "one of those neoplasms in which extrinsic factors are so important that they overshadow hereditary elements." Existing data thus suggest that if cancer of the uterus is influenced by genetic factors, it is cancer of the corpus uteri rather than cervical cancer.

Maternal Age and Birth Order. Factors of growth, development and senescence are known to have epidemiologic significance in a number of diseases and to exert that effect in respect to both mother and child[66]. Maternal age and birth order act in such non-infective processes as mongolism[51], congenital malformations[75b], cerebral palsy[61] and psychoneurosis[82].

An effect of maternal age has been demonstrated on several occasions to engender greater susceptibility of off-

conceivably involving cytoplasmic inheritance[104].

A possible association of maternal age and birth rank with cancer in man has scarcely been investigated. The frequent effect in other chronic diseases and in induced malignant neoplasia of experimental animals suggests such studies as useful in cancer of all sites, including cervix.

Co-existing Disease. Clinicians and epidemiologists alike have noted an association between cancer of the cervix and syphilis[18,60,64a]. A comprehensive study by Levin[60] of 5,610 white female cancer patients in New York State during 1940-41 demonstrated syphilis to be three times as frequent among women with cervical cancer as in women with cancer of other types (Table 4). Women with cancer of the fundus showed no association. Lombard and Potter[64a] found 8.4% of cervical cancer patients in Massachusetts

to be reactive to serological tests for syphilis but only 2.2% of those with cancer of the breast. The significance of these observations remains undetermined, whether that of a causal relation or that the same social factors within the environment which lead to syphilis also predispose to cervical cancer.

Hormonal Abnormalities. Ayre[2] demonstrated excess tissue estrogen in 46 of 50 patients with cancer of the cervix; and with few exceptions a vitamin B (thiamin and riboflavin) deficiency was also present. The interpretation was that with insufficient vitamin B, estrogens are not properly metabolized in liver, and hyperestrogenism follows. From this came the hypothesis that cervicitis results in increased concentration and fixation in cervical tissue of existing excess estrogens which in turn leads to carcinoma of the cervix.

Greene and Suckow[38] feel that if carcinoma of the cervix is caused by excessive amounts of endogenous estrogens the endometrium should become hyperplastic. Bainborough[3] reported hyperplasia of the endometrium in half of 26 cases of invasive cervical cancer collected over a 10-year period. Others refute these findings. Greene and Suckow[38] examined 11 endometrial specimens from cervical cancer patients and found only one with hyperplasia; Bayly and Greene[4] found only four instances of endometrial hyperplasia in 77 specimens; and in Speert's[99] study, endometrial hyperplasia was present in only 1 of 134 cases of carcinoma of the cervix. This led to the conclusion that the coincidence of cervical and endometrial lesions is obviously casual and without significance. Nieburgs[81] postulated that protracted low estrogen levels with resulting increase of pituitary activity might be etiologically significant.

Experimentally, malignant epithelial growths in cervix and vagina have been induced in various inbred strains of mice by a variety of estrogens[1,86]. Attempts to produce cervical cancer with estrogens in 47 rhesus monkeys, in experiments[89] continued over a period of 12 years, failed to produce a malignant neoplasm even with added and repeated trauma to the cervix by cauterization, biopsy and suturing.

The causal relationship between estrogens and endometrial carcinoma is also indefinite[83c] although endometrium reacts more to estrogenic stimulation than does the cervix.

Cervicitis and Lacerations. Cervicitis and traumatic lacerations are other gynecological conditions frequently advanced as predisposing to cancer of the cervix. Repeated examination of some 4,487 patients[12a,b] having had deep cauterization of the cervix after surgical operation for chronic tubal infection, cervical leukorrhea or other reasons led to recognition of cancer of the breast and of the fundus as anticipated for a general population, but cases of cervical cancer were only about one-seventh. The data collected were consistent with the hypothesis that cauterization reduced the expected incidence of cervical cancer, although not providing the final and unequivocal proof desirable.

Gagnon[32b] strongly endorses the concept that cervicitis stands in direct causal relationship with cervical cancer, because cancer of the cervix is much less frequent among nuns who by their mode of living are less exposed to factors causing cervicitis. Cancer of the cervix, as will be demonstrated, is rare among nuns, but to relate that primarily to absence of cervicitis is oversimplification. Many other differences exist between the life of women of the outside world and those in a convent, and a number have sug-

gestive relationship to cancer of the cervix.

Since cancer in general develops with some frequency in old scars, especially neoplasms of lip and skin, Levin[60] suggests that cancer of the cervix may also be an example of "scar cancer," particularly in syphilitic women. A causal relationship between unrepaired lacerations and cancer of the cervix was strongly supported by Lombard and Potter[64b].

Perhaps the issue is summarized as well as possible by Novak[83b] who recognizes "considerable differences of opinion among gynecologists as to the importance or unimportance of chronic irritative lesions as predisposers to cancer."

Nutritional Deficiencies. Much evidence has accumulated to indicate a relationship between particular dietary factors and cancer of experimental animals, especially cancer of the liver[105]. Whatever significance nutrition may have in cancer of the cervix probably rests in the effects of a general nutritional state on resistance and susceptibility of tissue. Cancer of the cervix occurs at more than usual levels among populations of lower social class; nutrition could be one of the basic factors which that observation indicates but does not identify.

PHYSICAL ENVIRONMENT. Material differences occur in the frequency of uterine cancer in different parts of the world[100a]. Deaths from cancer of uterus and of breast per 100,000 female population and for five widely scattered countries, United States, England and Wales, Denmark, Australia and Japan, are presented in Table 5. The differences are of sufficient magnitude to indicate that the causal complex responsible for cervical cancer is far from fixed, and in all probability varies in significant components from place to place.

TABLE 5.—CANCER OF UTERUS AND BREAST, DEATHS PER 100,000 FEMALES, AGE ADJUSTED TO 1950 FEMALE POPULATION, FOR FIVE SELECTED COUNTRIES; AND BIRTHS IN THOSE COUNTRIES PER 1,000 POPULATION, 1940

Country	*Year*	*Cancer of Uterus*	*Cancer of Breast*	*Birth Rate 1940*
United States	1950	21.2	24.7	17.9
Denmark	1948–49	20.1	25.6	18.3
England and Wales	1949	14.8	27.0	14.1
Australia	1946–48	15.5	24.8	17.9
Japan	1949	26.3	4.5	29.4

Source: WHO Epid. and Vital Statistics;[122b] National Office of Vital Statistics;[114a] Segi, M., Fukushima, I., Mikami, Y., Fujisaku, S., and Kurihara, M.;[98] U. N. Demographic Year book.[110]

Countries with high birth rates have been observed[103a] to have a greater proportion of cancer of the uterus than cancer of the breast, while the opposite holds for countries with low birth rates. Among the countries just listed, United States, England, Australia and Denmark had birth rates below 20 per 1,000 in 1940, and the frequency of breast cancer exceeded uterine cancer. Japan had a birth rate of 29.4 and uterine cancer predominated rather strikingly over breast cancer.

Rural-Urban Ratio. Cervical cancer occurs more frequently in urban than in rural populations. In the Iowa Cancer Morbidity Study of 1950 by the National Cancer Institute[41] the urban-rural ratio for cervical cancer was

1.77:1; for cancer of the corpus 1.23:1; and for breast cancer 1.25:1 (Table 6). Clemmesen and Nielsen[15c] also found cervical cancer to predominate in urban areas of Denmark and at every age. The observed differences are difficult to correlate with other knowledge of the disease. Better medical care in both diagnosis and treatment can reasonably be assumed to exist in cities and thus to contribute to an extent.

TABLE 6.—CANCER OF CERVIX UTERI, CORPUS UTERI AND BREAST, CASES PER 100,000 FEMALES, AGE ADJUSTED TO 1950 U. S. FEMALE POPULATION, URBAN AND RURAL, STATE OF IOWA, 1950

Cancer Site	*Total*	*Urban*	*Rural*	*Ratio Urban/Rural*
Cervix uteri	36.6	46.8	26.4	1.77
Corpus uteri	19.3	21.2	17.3	1.23
Breast (female)	70.3	78.0	62.4	1.25

Source: Haenszel, W., and Marcus, S. C.[41]

TABLE 7.—CANCER OF SELECTED SITES, CASES PER 100,000 FEMALES, AGE ADJUSTED TO 1950 U. S. FEMALE POPULATION, NORTHERN AND SOUTHERN CITIES, TEN-CITY STUDY, 1947. NORTHERN CITIES: CHICAGO, DETROIT, PHILADELPHIA, PITTSBURGH; SOUTHERN CITIES: ATLANTA, BIRMINGHAM, DALLAS, NEW ORLEANS

Race and Site	*Northern Cities*	*Southern Cities*
WHITE:		
Female genital	68.7	91.1
Female breast	69.0	70.0
NON-WHITE:		
Female genital	106.5	114.3
Female breast	56.1	51.9

Source: Dorn, H. F., and Cutler, S. J.[26]

Marriage is generally assumed to increase the risk of cervical cancer and there are more unmarried women in rural Denmark[14c] than in Copenhagen. The rural areas of Denmark and the wealthier parts of Copenhagen had an incidence about one-half that of the city as a whole, which leads Clemmesen to exclude "poor general hygiene" as a causative factor in cervical cancer.

North and South Latitudes. The Ten-City Study of the Public Health Service[26] demonstrates cancer of the cervix to be more frequent in cities of the south than in those of the north. The differences hold whether related to white or non-white fractions of the population (Table 7). Again, the reasons for these differences are troublesome to identify. Climate would seem rather clearly not a factor, although holding in such malignant growths as those of skin. Differences in social environment are the likely explanation, such matters as early marriage, more children, and other features of a lower economic status.

SOCIAL ENVIRONMENT. *Married and Unmarried.* Frequency of cancer of uterus and breast is a clearly defined function of marital status[25a,c,44,53,62]. As early as 1844, Stern[101] observed that the "married state increases the number of uterine (that is, cervical) cancers, while the single state and even more the monastic state increases . . . especially the number of mammary cancers." Reports from scattered sources on cancer among nuns are in

agreement with this observation and strengthen the case for existing differences in frequency of uterine cancer among married and unmarried women. Nuns in four Canadian convents[32a], with an average annual population of 3,280, in a period of over 20 years had no proven cases of cervical cancer, and only 2 deaths from cancer of the corpus uteri among 130 cancer deaths. Other observations in the United States and in Germany support the finding that cancer of the cervix is infrequent among nuns[96,107,108], this despite equally reliable information that total cancer among nuns[17,96] and monks[17] is at about the usual level for the general population concerned.

Recorded deaths by occupation in the Netherlands[113] for the years 1931 to 1935 give cancer of the cervix and corpus for nuns and members of religious orders as no less than expected deaths from these causes among other unmarried women.

Evidence is had that the relation between marital status and uterine cancer is more a matter of cancer of the cervix than of cancer of the corpus. Comparison of differing marital status of patients with primary cancer of the uterus in all London hospitals[44] during 1938-1939 gave a ratio of married and widowed to single women for cervical cancer of 25:1, and for cancer of the corpus 3:1. Among reported deaths in England and Wales, 1948-1949, cancer of the cervix was about twice as frequent among married women as in single women, while cancer of the corpus evidenced little difference. Breast cancer was more frequent in single women. Separating married women into fertile and infertile, based on whether or not they had borne children, Logan[62] computed equivalent average death rates for single women, married infertile, and married fertile, being respectively 62, 111 and 142. For cancer of the corpus no significant difference appeared although rates for women with children were somewhat less, the respective values as before being 39, 41 and 29 (Fig. 7). Logan concludes that married status of itself and apart from childbirth, seems to result in higher mortality from cervical cancer.

Marriage before 20 years of age was the most important finding in studies by Lombard and Potter[64a,b] designed to isolate social factors concerned with cancer of the cervix. Some 44.6% of cervical cancer patients were married before age 20 years, compared with 29.7% of matched controls. They interpreted this as indicative of some other variable directly related to cancer of the cervix, such as earlier childbirth, multiple children, poor obstetrical service, longer duration of married life, syphilis, immaturity of tissues at time of marriage or excessive hormonal stimulation, the latter on the assumption that girls of excessive sexual urge marry earlier than others. Presuming existence of an extrinsic carcinogen, exposure would be earlier and longer under conditions of early marriage. Irrespective of age at marriage, the married state furthermore remained significant after elimination of such variables as cervical laceration, parity and multiple children. This suggested to Lombard and Potter that some factor unconnected with childbirth exerted an effect, with an endocrine abnormality considered plausible.

Fertility and Childbearing. Analyzing Harnett's data[44] according to number of children born to eventual cancer patients in comparison with parity of the general female population of England and Wales, Stocks[103b] concluded that the risk of developing cancer of the cervix increased after three confinements, and that for women with five or more children was

twice that for women with one, two or three children. Cancer of the corpus was most likely to develop in married women with no more than one child. Dorn[25c] agrees that "physiologic changes associated with childbearing as well as marriage *per se* seem to play an important role."

An analysis of 1200 consecutive patients with cancer of the cervix at Cardiff Royal Infirmary led Maliphant[68] to conclude that after age 35 years the risk of developing cervical cancer was twice as great for married women as for single. If there were children, the risk was still greater.

Gilliam[33a] analyzed case histories of 2,497 patients from nine hospitals in three American cities and found that high birth rates under 25 years of age were a regular feature of patients with cervical cancer. He concluded that if childbearing is of etiological importance in production of cancer of the cervix, the effect is prior to the 25th year. A strong correlation between cervical cancer and the last child born to women before age 25 came from the

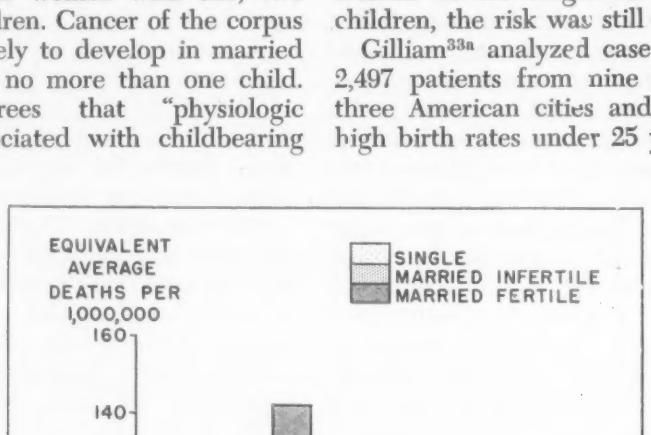

Fig. 7.—Equivalent average deaths per million from cancer of uterine cervix and body of uterus, females aged 15 years or more, England and Wales, 1948–1949; single, married infertile and married fertile.
Source: Logan, W. P. D.[62].

Lombard and Potter study[64b]; 24% of cervical cancer patients were in this category compared to 9% of controls, matched for age and economic status.

By adjusting number of pregnancies to age of first marriage in a study of hospital patients Wynder *et al.*[123] found the presumed significant difference in pregnancy rate between patients with cancer of the cervix and a comparison group to disappear. They conclude, therefore, that early age of coitus and not childbearing is the etiologic consideration.

Abortion and Miscarriage. These conditions have had attention of several workers as possible factors in cancer of the cervix[44,64b,123]. No satisfactory evidence of significance has developed, either one way or the other. Accurate information is obviously difficult to obtain. Because cancer of the cervix occurs more frequently in married than single women, and because abortions and miscarriages are also more frequent among the married, more such events would be expected among cervical cancer patients. Multiple marriage[123] and divorce or separation at any time[64b] have also been determined as positive associations.

Limited Sexual Intercourse. Jews, Moslems, Hindus and Parsees all have religious laws governing marital relations during menstrual and postpartum periods. These laws of behavior have been assembled in detail by Kennaway[54]; only the main features are given here.

Abstention from intercourse by Jews during menses is 12 days from onset of flow, almost half of the ovulatory cycle. The postpartum period of uncleanliness is prescribed as 40 days following birth of a male and 80 days for a female child.

Hindu religious law prohibits marital relations for four days during the menstrual period, during which women are isolated and subject to numerous other restrictions. Parsee law regarding menstruation is much the same as for Hindus. The Koran directs Moslems to "separate themselves from their wives during their courses," but with no stipulated time. These customs provide a background for interpreting results of studies of cancer of the cervix in the particular groups.

Cancer of the cervix is infrequent among Jewesses. Kennaway[54] reviewed this extensively, using information from whole countries and cities and at other times from admissions to one or more hospitals. Data were rarely comparable and many times cancer of cervix and corpus could not be separated, but invariably a lower frequency of cancer of the uterus characterized Jewish as compared with non-Jewish women. Recent studies in the United States are on the basis of separate consideration of cancer of corpus and cervix. The limited frequency of uterine cancer is seen to be regularly due to comparative rarity of cancer of the cervix. Squamous cell carcinoma of the cervix is even rarer among Jews than adenocarcinoma[40]. Of 999 patients with squamous cell carcinoma of the cervix in New York[18] three were Jews, a ratio of 1:333; of 48 patients with adenocarcinoma of the cervix, six were Jewish women, a ratio 1:8.

All cases of uterine carcinoma at Beth Israel Hospital, Boston, from 1928 to 1953 (Table 8), gave a ratio of cervical cancer to corpus among Jewish women of 1:4; and for non-Jewish women 2:1. In a series of 239 ward patients with cervical cancer admitted to Mount Sinai Hospital[118], New York, 1928 to 1948, 189 were non-Jewish and 50 were Jewish, to give a ratio of 5:1 when adjusted for total numbers of Jewish and non-Jewish in the general hospital population.

These differences have been explained variously on the basis of genetic resistance[68], hygienic practices dictated by religious regulation[54], circumcision of Jewish males in infancy[42b] and a special hormonal state of Jewish women[14c]. Inability to reconcile other existing host and environmental factors is always a difficulty. A promising approach is the concurrent observation of Jewish women living in Israel and others resident in New York City[106] which is now underway.

SOCIAL STATUS. British data for 1930-1932 indicate an inverse frequency of cancer of the uterus according to social level[54,92]. In Copenhagen[15a] (Fig. 8), cancer of the cervix but not of corpus uteri tends to occur more often in poorer classes, among both married women and women never married. Breast cancer of women showed an evident

TABLE 8.—CANCER OF UTERUS, CERVIX AND CORPUS, PER CENT DISTRIBUTION OF CASES, JEWISH AND NON-JEWISH WOMEN, BETH ISRAEL HOSPITAL, BOSTON, 1928–1953

Site	*Total Cases*	*Jewish*		*Non-Jewish*	
		Number	*Per Cent*	*Number*	*Per Cent*
All cancer of uterus	173	93	100	80	100
Cervix	71	19	20.5	52	65.0
Corpus	102	74	79.5	28	35.0
Ratio cervix:corpus		1:3.9		1.9:1	

Source: Ober, W. B., and Reiner, L [84]

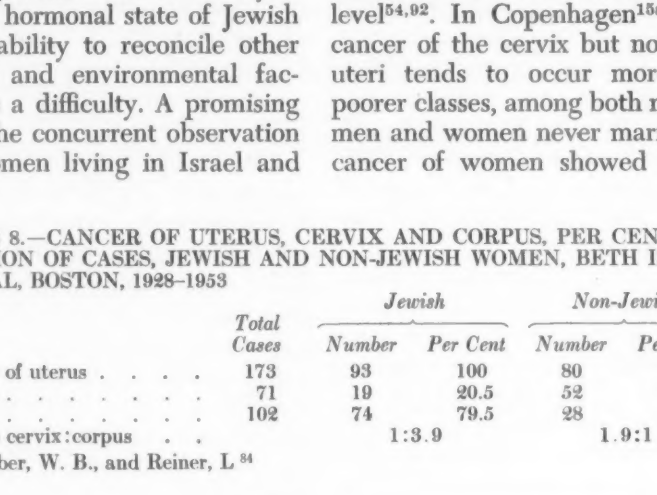

Fig. 8.—Cancer cases, female, incidence by selected sites and by average rental, Greater Copenhagen 1943–1947; category 1 is highest rental paid; 5 lowest.
Source: Clemmesen, J., and Nielsen, A.[15a].

tendency in the opposite direction. Lung carcinoma of males simulated behavior of cervical cancer in women.

In Pittsburgh areas[88] of lowest economic status, genital cancer of white females was 60% above the average for the city. Conversely, white women in areas of highest social status had twice as much cancer of the breast as those living in other parts of the city.

Low social and economic state of itself is not a direct causative factor in cervical cancer or in any other disease. That condition does provide an index of where to look for causative factors and much indication of what they might be. Since cancer of the cervix has a high level of occurrence among low economic groups, attention is thus directed to factors associated with that state, such as earlier marriage, more children, multiple marriages, fewer circumcisions, poorer hygienic conditions, less satisfactory obstetrical care, and perhaps more syphilis, all of which may lead to more cervicitis, trauma or whatever else is the direct cause. The greater frequency of cervical cancer among Negroes than whites in the United States may well rest on differences in social environment rather than racial considerations. Conceivably the social component of environment may also be responsible for the observed differences in cervical cancer in India among Deccani Hindus contrasted to Moslems. Social state is no satisfactory explanation for infrequency of the disease among Jews; that relation holds irrespective of differing social levels, in all of which Jews are represented.

THE EPIDEMIOLOGIC PROBLEM. The business of epidemiology being diagnosis of mass disease, the obligation now is to strike a balance, to establish the position of cancer of the cervix as a problem of the public health. The first requisite, to confirm the condition as a mass disease of human populations of such nature and magnitude as to warrant responsibility by community health agencies, may be taken as settled. The niceties in diagnosis, which permit full command of a situation and direct specific administrative action, have not been so satisfactorily resolved.

Before remedy can be introduced, cause must be known; and before cause can be determined the nature of the mass disease must be understood. The first need in cervical cancer, is to know the varying frequencies in different fractions of a population, the characteristics of persons affected and the more meaningful features of the environment. This is basic epidemiologic analysis. A number of these items have been determined with much assurance, others less definitely.

With the fundamentals in hand, suspected causative factors are to be tested to determine whether or not their presence is consistent with occurrence of the disease in populations. Current hypotheses on causation of cervical cancer are of two main classes: the one recognizes the host as making the main contribution through hormonal or other influences; the other lays stress on a postulated agent of disease, an extrinsic factor of the environment, a carcinogen. Lack of quantitative information on occurrence in a general population both of disease and factors in question, is a main difficulty in developing these suggested explanations. Frequency distributions among hospital patients, the separation of tumors by pathological means, or deaths from the prescribed condition do not provide that kind of information. It is to be had by field study. The direction of epidemiological effort is conceivably along two general lines.

In common with most chronic disease, a long term field investigation

of cervical cancer, with observations extending over many years, is believed to be the approach promising most definite answers. As expensive in material resources and as exhausting of time as such investigations are, the ultimate answer to this and many other problems of its kind is seemingly through this longitudinal technique. There are good reasons for this opinion. In slowly developing organic disease, the emergence of causative relationships must be anticipated as far slower than in germ-caused epidemics. There is the further primary need to relate inferences on causation to populations rather than groups of patients.

A second general class of epidemiologic studies has the aim of intensive investigation to test selected hypotheses of causation, whether originating in clinical, laboratory or field investigation. Many exist, as this review has demonstrated; others may be expected from the approach just outlined. The circumcision question, the resistance of Jewish women, infections of the vaginal tract, and the association in populations of penile and cervical cancer are in the forefront. Marital history, associated precancerous conditions including carcinoma-in-situ, and the hormonal abnormalities of women warrant serious attention. This approach is in accord with the research axiom to center effort on specific objectives having definite suggestion of result rather than attempt to catalogue all happenings with the risk that the relevant is obscured by the irrelevant.

The development and test of field methods applicable to recognition of cervical cancer in general populations is a necessity, because existing programs for control rest almost wholly on treatment of early disease by clinical methods. The main function of the field worker under such circumstances is to maintain the stream of suspicious cases to clinical facilities. Desired qualities of such a field diagnostic method are simplicity, cheapness and a reliability not too wasteful in costs of confirmation.

A better definition of incidence and prevalence is also necessary if epidemiology is to fulfill its second function of prognosis, the probable movement of cervical cancer in populations by time and place. The reasonable forecast is for a greater problem. Even if attack rates remain as they are, numbers of persons with cancer of the uterus will increase because of an aging and a larger population. Greater rates are also a reasonable projection because of such artificial stimuli as the required registration of cases by hospitals and an improved clinical practice; also by actual increase, through augmented host stresses and greater activity of such carcinogens as may be proved to exist.

CONTROL OF CERVICAL CANCER. Other than for subsequent evaluation of what has been accomplished, the obligation of the epidemiologist ends when the public health administrator is provided with the facts upon which to operate. The benefit to a beleagured population comes from what public health practice is able to make of these facts in developing a program for control. In the attack on mass disease, public health practice like therapeutics in clinical medicine directs effort along two lines, that of prevention and that of limiting the effect from already existing disease. Prevention is the fundamental consideration; cure or amelioration are at best temporizing or emergency measures so far as control is concerned, and that holds whether the unit of observation is a person or a population.

In therapeutics the primary effort of the clinician is to cure disease; its counterpart in mass disease is to eradi-

cate the condition from the population involved. For cancer of the cervix, the clinician finds measurable promise of success in attempted cure; the public health administrator has no prospect of accomplishing eradication now or in the foreseeable future. As a consequence, emphasis in the mass disease is on that secondary objective which clinical and mass medicine have in common, the attempt to limit defect and disability where cure is impossible. Present day control of uterine cancer is largely by these measures. Preventive practices are few, and general rather than specific. Despite these restrictions, the progress to be reported in the forthcoming description of incidence and mortality from uterine cancer in Massachusetts[53] is seen to be material.

Because most of what constitutes practical cancer control lies in early recognition of the disease and prompt treatment, the provision of diagnostic facilities to physicians has a prominent place in programs. A service for histologic diagnosis of biopsy and other specimens is maintained by a number of states; provision for cytologic examination of vaginal smears is less common.

To the same end, organization of training facilities for private physicians with emphasis on case-history presentations and opportunity to become familiar with techniques of physical examination and newly developed laboratory aids are definitely in point.

The growing practice among hospitals of maintaining cancer registries is to be encouraged by public health agencies, with more use of the information in assaying the cancer problem of the community.

The provision of state aid to hospitals, for care of patients lacking ability to obtain adequate medical service, is a prominent feature of the cancer control program of numerous states.

The official health agency in its cancer control work has the principal obligation to assure that groups at high risk have the advantage of periodic physical examination and that persons with suggestion of cervical cancer have the benefit of early medical attention. The approach is by identification of groups at high risk by field survey, the addition of educational measures on the nature of cervical cancer by public health nurses in the course of visits for other purposes, and general public health education on danger signals of cervical cancer and the importance of acting promptly in seeking aid from a physician.

The difficulty with general public health education in this field is that so little of demonstrated fact is at hand upon which to base reliable preventive recommendations. Effort toward a better practice of sexual hygiene among a general population is doubtless a good thing, but little result is to be expected until it can be specifically directed. The attack on filth in the early nineteenth century was laudable but without great result until effort could be centered on contaminated water, infected rats and dangerous food practices. Early marriage and the practice of circumcision have been associated with more and with less cervical cancer and yet for both the proof of a causal relation is indefinite and the universality of the connection ill-defined. They stand in the position of so many preventive measures, suggestively worthwhile, justifiably endorsed by the private physician to fit a particular circumstance, but scarcely possessing the certainty and general applicability which the public health physician requires when he speaks to a general population.

With central effort primarily in measures to limit a disease already present, and with so few available preventive measures, the activities of cancer control units in cervical cancer are strongly directed toward research. Remedial measures being the main reliance, clinical investigation to bring them to highest efficiency is clearly indicated. Field study by epidemiologic methods has the aim to identify those fractions of a population of greatest susceptibility, and thereby promote early medical attention, consonant with demonstrated need. The more basic objective of a search for causes of such distributions gives attention not alone to relative importance but to the practicability of corrective measures which are economically feasible and socially acceptable. Laboratory investigation continues first, however, in the search for cause.

The epidemiologic method has the further application in control programs, of evaluating newly instituted procedures or test of older methods of approach. A properly designed pilot study, with analysis of results, will often conserve funds and effort, and avoid unwarranted discredit to general cancer control.

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BOOK REVIEWS AND NOTICES

BIOCHEMICAL DETERMINANTS OF MICROBIAL DISEASES. By RENÉ J. DUBOS, The Rockefeller Institute for Medical Research. Pp. 152; 3 ills., 49 tables. Cambridge, Mass.: Harvard University Press, 1954. Price, $3.50.

THIS small volume is a collection of lectures given in Boston and in California during 1953 and 1954. Although they lack some degree of continuity, there is throughout a central theme which is pursued with vigor. And that theme is this: Heretofore the study of infectious disease has concentrated upon the microbial parasite to the neglect of the host. It is now time to work more upon the host factors so that the parasite will have no chance to cause disease. For the time being Dr. Dubos confines himself to the consideration of certain biochemical factors known to retard or to promote microbial proliferation *in vivo*.

No doubt some readers will be of the opinion that the author has cited the work of his own group to the exclusion of that of other investigators. But the fact is that Dr. Dubos, Pasteur's most imaginative disciple and possibly the outstanding American bacteriologist, has scored another bullseye.

E. S.

NERVE BLOCKS. By JOHN ADRIANI, M.D., Director, Department of Anesthesiology, Charity Hospital of Louisiana; Professor of Surgery, Tulane University of Louisiana School of Medicine. Pp. 265; 168 ills. Springfield, Ill.: Charles C Thomas, 1954. Price, $6.50.

DR. ADRIANI describes the anatomy, techniques, indications, contraindications, and complications for the more commonly used nerve blocks. His presentation is brief, compact, and comprehensive.

The sketches and photographs are excellent and should be a great aid to the beginner in the study of regional anesthesia. Included in the general consideration of regional anesthesia, the author describes the physical, chemical and physiological properties of the sixteen most commonly employed local anesthetic drugs.

The text includes a most complete description of the following regional anesthetic procedures: paravertebral block anesthesia, blocks of the cranial nerves, blocks of peripheral nerves, field blocks, spinal anesthesia and epidural anesthesia.

With the exception of spinal epidural anesthesia, Dr. Adriani has performed his usual excellent work. The description of the technique for spinal epidural anesthesia is sketchy and rather incomplete. With the renewed interest in epidural anesthesia, Dr. Adriani could have referred to some of the more recent concepts of spinal epidural anesthesia.

This textbook is certainly a worthwhile contribution to the specialty of anesthesiology and should be recommended to the beginners in anesthesiology.

L. K.

ADVANCES IN PROTEIN CHEMISTRY, VOLUME VIII. Edited by M. L. ANSON, KENNETH BAILEY AND JOHN T. EDSALL. Pp. 529; 60 ills. New York: Academic Press, Inc., 1953. Price, $10.50.

ADVANCES are presented for seven fields of protein chemistry. They include discussions of possible modes of synthesis of proteins from naturally occurring peptides; peptide bond formation; the nature and reproduction of bacteriophages; assimilation of amino acids by bacteria and the effects of antibiotics; the nutritional and non-nutritional uses of peanut protein; the physical basis for the phenomena of rotational Brownian motion and polarized fluorescence of solutions; and a valuable article on zone electrophoresis for beginners in this field.

J. B.

AN INTRODUCTION TO PATHOLOGY. By G. PAYLING WRIGHT, D.M., F.R.C.P., Guy's Hospital Medical School. 2nd ed. Pp. 636; 248 ills. New York: Longmans, Green & Co., 1954. Price, $7.50.

THE title of this book denotes its content precisely. Dr. Wright is professor of pathology at Guy's Hospital Medical School and he has written this text for his students who are beginning their courses in this subject. It is not to be compared with the numerous American textbooks in pathology since it deals only with that phase which in the latter is usually designated "General Pathology" found in the first quarter of these texts. In the English curriculum, Systemic Pathology is taken up in later courses.

As a book in "General Pathology" it is an admirable example which many of the Amer-

ican authors might do well to strive to equal. In the simplest and broadest terms he describes the general principles of his subject paying the greatest attention to the aspects of etiology, and morbid physiology though he does not neglect the structural changes of diseased tissue. Any student with a comprehensive knowledge of the contents of this volume should find his "Systemic Pathology" a far more significant and rewarding course than the average American student who has been hurried through his early training in order to have sufficient time for the pathology of specific disease entities. It is, perhaps, a more leisurely approach to the subject but it seems to this reviewer that it is the more sensible one, and possibly, in the end, no more time will have been consumed. I would prophesy that eventually, a number of American schools will adopt this method of teaching this most important course.

E. A.

SEROLOGICAL APPROACHES TO STUDIES OF PROTEIN STRUCTURE AND METABOLISM. Edited by W. H. COLE. Pp. 97; 46 ills. New Brunswick, N. J.: Rutgers University Press, 1954. Price, $2.00.

THE material included in this report was presented at the Tenth Annual Conference on Protein Metabolism of the Rutgers University Bureau of Biological Research. The present conference was concerned primarily with the nature of the precipitin reaction rather than with its clinical applications. The subjects discussed included: 1) Proteins as Antigens and Antibodies; 2) The Precipitin Reaction and Studies of Native and Denatured Proteins and Derivatives; 3) Light Scattering and Ultracentrifugation Methods in Studies of Precipitin Systems and Their Application to Human Metabolism; 4) Immunolgical Analysis of the Biosynthesis of a Protein; 5) Use and Limitations of Serum-Agar Techniques in Studies of Proteins; and 6) Measurement and Significance of Serological Correspondence Among Proteins.

The volume will be of particular interest to immunochemists, serologists, and bacteriologists.

C. Z.

NEW BOOKS

Newer Concepts of the Causes and Treatment of Diabetes Mellitus. Pp. 181; 43 ills. New York: The National Vitamin Foundation, Inc., 1954. Price, $2.50.

THIS book presents a series of reports given in a symposium conducted by the New York Diabetes Association in October, 1953. The arrangement is such that the first portion of the Proceedings deals with the fundamental biochemical advances and recent experimental data in diabetes mellitus; the final sections are devoted to clinical applications of this newer knowledge. Among the contributors to the first part are some of the outstanding investigators in this field: Mayer discusses The Hereditary Obese-Hyperglycemic Syndrome in the Mouse; Gurin, Lipogenesis in Diabetes; Long, The Endocrines and Carbohydrate Metabolism; Houssay, Hormone Interrelationships; Stadie, The Action of Insulin; and Wrenshall, Glucagon. Equally imposing is the list of authors whose long clinical experience and observations lend weight to their views on the current methods and concepts of the treatment of diabetes mellitus. Although the latter section of the book does not provide details of the methods employed in treatment the broad general concepts which are discussed here will provide the informed practitioner with knowledge useful in the management of his patients. This symposium should be read by all physicians interested in this disease and its treatment.

C. S.

The Mentally Subnormal Child. Report of a Joint Expert Committee convened by WHO with the participation of United Nations, ILO, and UNESCO. WHO Technical Report Series, No. 75. Pp. 46. Geneva: World Health Organization, 1954. Price, $.25.

Expert Committee on Environmental Sanitation. Third Report. WHO Technical Report Series, No. 77. Pp. 25. Geneva: World Health Organization, 1954. Price, $.25.

Expert Committee on Rheumatic Diseases. First Report. WHO Technical Report Series, No. 78. Pp. 25. Geneva: World Health Organization, 1954. Price, $.25.

Expert Committee on Rabies. Second Report. WHO Technical Report Series, No. 82. Pp. 27; 3 ills. Geneva: World Health Organization, 1954. Price, $.25.

Smallpox Vaccination. A survey of recent legislation. Pp. 41. Geneva: World Health Organization, 1954. Price, $.50.

Treponematoses. A World Problem. BY T. GUTHE and R. R. WILLCOX. Pp. 77; 27 ills. Geneva: World Health Organization, 1954. Price, $.50.

A Primer of Pulmonary Function. By HAROLD GUYON TRIMBLE, M.D., and JAMES KIERAN, M.D. Pp. 22; 1 ill. Oakland: California Tuberculosis and Health Association, 1954. Price, not given.

Nerve Impulse. Transactions of the Fourth Conference, March 4–6, 1953. Edited by DAVID NACHMANSOHN, M.D., Columbia University College of Physicians and Surgeons. Pp. 224; 54 ills. New York: Josiah Macy, Jr. Foundation, 1954. Price, $4.00.

THE fine quality of this report is maintained in this current edition. The contributors continue to be the outstanding authorities in their fields. This volume continues to be a prime source for material for workers in the field. M. O.

Clinical Chemical Pathology. By C. H. GRAY, D. Sc., M.D., Prof. of Chemical Pathology, University of London. Pp. 138; 17 ills. London: Edward Arnold & Co., 1953 (Williams & Wilkins Co., Baltimore, U. S. Agents). Price, $3.00.

THIS small volume is based on a series of lectures in chemical pathology given by the author to medical students at King's College Hospital Medical School. The various topics which are traditionally studied in medical school courses in biochemistry are presented in a simplified manner. Accordingly, medical students and practitioners may find this book of some value for a brief review in the subject. C. Z.

Causality in Natural Science. By VICTOR F. LENZEN, PH.D., Professor of Physics, University of California. Pp. 121. Springfield, Ill.: Charles C Thomas, 1954. Price, $3.00.

THIS is an interesting and stimulating philosophical discussion of causality which involves a knowledge of physics not possessed by most physicians. The concept of cause and effect commences with man's personal experience that a stone feels heavy. Then a god of lightning is devised in man's image. A soul is ascribed to a magnet when it behaves as a compass. Observation defines force as mass times acceleration. Causality comes to mean efficacy, uniformity or identity and eventually a relationship of phenomena without implication of cause and effect. Chapters discuss causality in biology and the relativity and quantum theories. M. O.

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